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# Response of Ectomycorrhizal Fungal Fruiting to Nitrogen and Phosphorus Additions in Bartlett Experimental Forest, New Hampshire

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RESPONSE OF ECTOMYCORRHIZAL FUNGAL FRUITING TO NITROGEN AND  
PHOSPHORUS ADDITIONS IN BARTLETT EXPERIMENTAL FOREST,  
NEW HAMPSHIRE

By  
Claudia N. Victoroff

A thesis  
submitted in partial fulfillment  
of the requirements for the  
Master of Science Degree  
State University of New York  
College of Environmental Science and Forestry  
Syracuse, New York

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## Abstract

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Forest productivity and recovery is limited by nutrients including nitrogen and phosphorus. Ectomycorrhizal fungi (EMF) form mutualistic symbioses with trees and aid roots in acquiring soil nutrients. The composition of EMF in forests may be sensitive to changes in soil nutrients in ways not fully understood. This research investigates EMF fruiting responses to nutrient manipulation in a project on Multiple Element Limitation in Northern Hardwood Ecosystems where N and P have been added annually in a factorial design since 2011. Sporocarp abundance, biomass, species richness, and fruiting community composition were compared between nutrient addition plots and control plots. While some ectomycorrhizal fungi are known to respond to N fertilization, this work is among the first to observe sporocarp community response to P fertilization, and to N and P fertilization together, which will be important to predicting how fungal communities will respond to changing soil nutrient conditions in a changing world.

Key Words: ectomycorrhizal fungi, community ecology, forest ecology, nitrogen, phosphorus, nutrient limitation

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# Chapter 1: Introductory Literature Review

## INTRODUCTION

The aim of this chapter is to provide context for the research discussed in chapter 2. This chapter provides a history on the study of symbiosis and mycorrhizae and an overview of mycorrhizal ecology with a focus on the fungi which form ectomycorrhizae. Literature on the responses of these fungi to changes in forest nutrient conditions will be examined.

### *The History of Symbiosis*

In 1842 Carlos Vittadini observed the husk of tree feeder rootlets enclosing mature sporocarps of the fungal genus *Elaphomyces*, and hyphae from the fungus surrounding rootlets with no sign of tree disease (Trappe 2015). Vittadini hypothesized that the fungus was nourishing the tree roots and thus was the first scientist to suggest that a fungus could be anything besides a decomposer or a parasite. While Vittadini's assertion was novel, it was largely overlooked, and he did not pursue the topic again (Trappe 2015). The introduction of the concept of symbiosis is often credited to German botanist and mycologist A.B. Frank who described the regular coexistence of two dissimilar organisms in his essay, *Über die biologischen Verhältnisse des Thallus einiger Krustenflechten*, or loosely, *On the biology of the Thallus of some crust lichen* (Frank 1877). In this essay Frank describes the development of lichenized fungi from the germination of the spore to the maturation of the thallus (Frank 1877). More importantly, Frank identified the need for a neutral term to describe the coexistence of two or more organisms regardless of the roles of those organisms in the interaction nor the necessity of that interaction for the survival of any one participating organism (Frank 1877). For this purpose, Frank recommended the term

‘symbiotismus’ and cited a range of examples of interactions between organisms which could be considered ‘symbiotismus’ (Frank 1877). The lowest level of symbiosis, according to Frank, is parasitism and the highest level is ‘homobium’. By Frank’s own definition homobium is a case in which dissimilar organisms unite to form another simple individual (Frank 1877). Homobium, perhaps, is the term Frank would suggest describing most lichens.

Frank was amongst the first to observe and describe various examples of symbioses including lichens and root nodules on legumes, and his 1885 depictions of the development and ecology of ectomycorrhizae rival the accuracy and detail of modern illustrations (Trappe 2005). Frank’s observations led him to formulate bold hypotheses about mycorrhizal fungi that contradicted the botanical wisdom of his age (Trappe 2005). Many of Frank’s initial hypotheses were supported through subsequent experiments (Frank 1885b, c, 1887a, 1888, 1889, 1891, 1892, 1894).

In 1878 the German botanist H.A. de Bary utilized the term ‘symbiosis’ to describe a number of interactions between dissimilar organisms in his speech entitled, *Die Erscheinung der Symbiose* or *The phenomenon of symbiosis* (de Bary 1878, translated in Oulhen *et al.* 2016). Importantly, de Bary cited the occurrence of symbiosis as an obvious and observable example of evolution, noting that the theory of evolution can explain the very occurrence of symbiosis:

“We have ample reason to agree with Darwin to say that successive adaptations and the correlating changes of morphology and transformations of organisms occur, and must occur, as a consequence of the influence of the environment on the organisms and on their capacity for transformation... Evidences to support the fundamental theory [of

evolution] that we have talked about are found everywhere. We just have to carefully look around.”

Not 20 years after the original publication of Charles Darwin’s *On the Origin of Species*, de Bary recognized symbiotic relationships as not only examples of evolution but as observable support for the theory of evolution (de Bary 1878, translated in Oulhen *et al.* 2016). Further, de Bary’s lecture was foundational in the recognition of symbiotic relationships, especially those involving microorganisms. Like Frank, de Bary described examples of symbiotic relationships ranging from mutualistic to parasitic. de Bary introduced a gradient of possible interactions between microorganisms ranging from parasitic relationships in which one organism quickly kills its host to mutualistic interactions in which each organism supports the fitness of the other.

Since the introduction of these formative ideas by Vittadini, Frank, and de Bary, the use of the word ‘symbiosis’ has been used increasingly synonymously with ‘mutualism’. The use of the term ‘symbiosis’ to describe exclusively mutualistic interactions contradicts the intended use of the word by both Frank and de Bary. I consider the use of the word ‘symbiosis’ as a synonym for mutualism to be inappropriate and consider any regularly occurring interaction between dissimilar organisms to be, definitively, symbiotic.

An array of microorganisms interact with plants in symbioses. In both historic and contemporary research on microorganisms, considerable attention is devoted to the study of pathogenic microorganisms that are detrimental to valuable horticultural or forest species. However, increasing attention and research funds are devoted to the study of microorganisms forming symbioses that might benefit such valuable plant species. Our understanding and value of many of these microorganisms is often determined by the impacts that these symbioses have on

the health and performance of profitable plant species but this human bias has historically limited the scope of research (Kaishian and Djoulakian, unpublished). Fungi perform vital ecosystem functions and influence humanity in diverse ways (Mueller and Bills 2004), yet our understanding of the ecology and biodiversity of fungi is lacking relative to other kingdoms. A lack of clear information on fungal biodiversity, due in part to a relative lack of fungal taxonomists, limits our ability to describe the full breadth of ecological roles that fungi fill and has implications for conservation, land use planning, and plant and animal pathology (Mueller and Schmit 2007; Fisher *et al.* 2012).

Fungi are estimated to be amongst the most diverse group of organisms on earth. In David Hawksworth's landmark 1991 paper he estimated the existence of 1.5 million species of fungi. This estimate was based on the ratio of fungal species relative to vascular plant species within the British Isles and was extrapolated to different regions of the world. Hawksworth considered this estimate to be conservative, in part because it did not make amendments for the possibility of higher ratios of fungi to plants in tropical or polar regions (Hawksworth 1991). Hawksworth and Lücking (2017) amended this estimate and arrived at 3.8 million species. Meredith Blackwell's estimate of fungal diversity in a paper entitled "The Fungi: 1, 2, 3 ... 5.1 million species?" reflects the increased rate at which new fungi have been described since the common application of molecular identification methods. When Hawksworth published his estimate in 1991 there were about 69,000 described species of fungi, but between 2008 and 2011 that number increased to about 99,000 described species. Blackwell projects that current and future molecular techniques will empower mycologists to describe the world's unknown fungi in the next 1000 years (2011). By either Hawksworth's 1991 or 2017 estimates, or Blackwell's 2011 estimate, it is clear that a small portion of the world's fungal biodiversity has been described by science. Without a clear

concept of fungal biodiversity our concept of fungal ecology suffers as well. I suspect that as fungal species are named and described new questions will arise regarding the role that endemic and cosmopolitan species play across different ecosystems, and how those roles may change in a changing climate.

The vast diversity of microorganisms is based, in part, on their adaptability to exploit a range of ecological niches. Fungi can derive energy from living, nonliving, or dead substrates and can exist in extreme environments including arctic regions, plant-free high alpine zones, and the deep sea (Bridge and Spooner 2012; Schmidt *et al.* 2012; Nagano and Nagahama 2012). Fungi perform many roles in ecosystems and often facilitate basic ecosystem functions (Smith and Read 2008). Fungi fill multidimensional functional niches (Lilleskov *et al.* 2015), so to categorize them as only mutualists, decomposers, or parasites is to limit our understanding of the extremely diverse roles that they play within ecosystems. Although phylogenetic diversity patterns can often be correlated with ecological functional diversity, there is a misconception that similar fungal taxa will *necessarily* function similarly in ecosystems. In reality, two different species within a genus or even two different individuals within a species may function differently due to genetic variation or under different ecosystem conditions (summarized by Diaz and Cabido 2001). Alternatively, phylogenetically dissimilar taxa may share functional traits (Parrent *et al.* 2010). Fungal species can be conspicuous; forming large and often colorful sporocarps, or subtle; existing totally microscopically such as the Glomeromycota or embedded within their substrate such as the endophytic fungi. Importantly, the conspicuousness of a species is not indicative of the magnitude of its role in an environment. As previously unknown fungal species are named and described by science our understanding of their roles within ecosystems becomes clearer. Because diversity is composed of both biodiversity and functional diversity (Diaz and Cabido 2001), we cannot divorce

our study of the phylogeny of these organisms from their ecology nor can we divorce our ecological research from organismal phylogeny.

### *Introduction to Mycorrhizal Ecology*

Mycorrhizae, or the exchange of resources at the interface of fungal hyphae and plant roots, are Earth's most prevalent symbiotic relationships in terrestrial systems (Smith and Read 2008). The basic exchange of resources in mycorrhizal symbioses involves the absorption and supply of soil nutrients by the fungus to the plant for a share of the plant's photosynthetically derived carbohydrates (Smith and Read 2008). These mycorrhizal associations occur in almost all ecosystems and most plants form mycorrhizae (Smith and Read 2008; van der Heijden *et al.* 2015). The word 'mycorrhiza' is a combination of the Greek roots "myco", meaning fungus, and "rhiza", meaning root (Frank, as cited in Trappe 2005). A. B. Frank hypothesized that mycorrhizae represent pervasive and mutualistic symbioses in which fungal hyphae absorb and transport mineral nutrients to plant roots and in turn are nourished by photosynthetically derived carbohydrates from the plant (Frank, as cited in Trappe 2005). This theory was controversial in its infancy as it contradicted much of the existing botanical paradigm (Trappe 2005).

While mycorrhizal symbioses are often considered mutualisms, they are dynamic relationships that vary based on the plant and fungal species involved and on the environmental pressures present in an ecosystem. Under shifting environmental conditions or when a new pressure, such as a drought or soil pathogen, arises in an ecosystem, mycorrhizal partnerships may increase the resiliency of that system (Pickles and Simard 2017). A mycorrhizal partnership may be considered mutualistic when the interaction is a net benefit to the plant and parasitic when the net cost of the interaction exceeds its benefits to one of the symbionts (Johnson *et al.* 1997).

Mycorrhizal relationships between fungal and plant partners can exist in all iterations of symbioses along the mutualism-parasitism continuum (Johnson *et al.* 1997). Mycorrhizal fungi may provide up to 80% of a plant's required nitrogen and 90% of required phosphorus (van der Heijden *et al.* 2008). There are four main types of mycorrhizal associations including arbuscular mycorrhizae (AM), ericoid mycorrhizae, orchid mycorrhizae, and ectomycorrhizae. These four types are categorized mainly by the morphology of mycorrhizal structures as determined in most cases by the plant partner. Some fungal groups may form different mycorrhizal types with different plant species (Vrålstad *et al.* 2002a).

Fungi involved in arbuscular mycorrhizal associations belong to the phylum Glomeromycota and depend entirely on plant hosts for carbon compounds. Arbuscular mycorrhizal associations facilitated nutrient uptake by early land plants allowing previously aquatic lineages to occupy terrestrial systems (Pirozynski and Malloch 1975; Dotzler *et al.* 2009). Fossil spores that closely resemble those of Glomeromycota have been described from the Ordovician (455-460 Ma) (Redecker *et al.* 2000). Fossilized AM structures within plants cells have been found from the Rhynie Chert formation, an early Devonian sediment deposit, suggesting that AM symbiosis occurred over 400 million years ago (Remy *et al.* 1994; Taylor *et al.* 2004; 2005; Taylor *et al.* 1999; Phipps and Taylor, 1996). Berbee and Taylor (2010) suggested that Glomeromycota arose earlier than this fossil evidence suggests and likely pre-date terrestrial plants. AM associations are characterized by the presence of intracellular hyphae which penetrate the cell wall of plant roots, extraradical mycelium which mine for soil nutrients, and spores which germinate asexually from hyphae. It has been estimated that over 80% of all vascular plants form arbuscular mycorrhizae, including some gymnosperm families and most angiosperm families. Many important horticultural plants form arbuscular mycorrhizae.

Ericoid mycorrhizae are characterized by the colonization of epidermal cells and the formation of hyphal coils in the epidermal cells of fine hair roots of plants in three families within the order Ericales. These families are Ericaceae, Epacridaceae, and Empetraceae. Plants that form ericoid mycorrhizae grow typically in areas with nutrient-poor soils suggesting that the fungi specific to ericoid mycorrhizae play an important role in nutrient acquisition for these plants (Peterson *et al.* 2004). Fungi involved in ericoid mycorrhizae mostly belong to the phylum Ascomycota though some fungi within the phylum Basidiomycota also form these associations. Some fungal species that form ectomycorrhizal associations will form ericoid mycorrhizal associations with members of the plant order Ericales (Vrålstad *et al.* 2002a). Members of Ericaceae that are considered commercially important are mainly in the genera *Vaccinium* (including blueberries and cranberries), *Erica*, and *Rhododendron*.

Orchid mycorrhizae occur exclusively within the plant family Orchidaceae, which is the largest family of flowering plants. Unlike other types of mycorrhizal symbioses, which are generally considered mutually beneficial to both the fungal and plant partners, orchid mycorrhizae have little to no known benefit to fungal partners and can be considered parasitic relationships (Smith and Read 2008). Orchid mycorrhizae are characterized by the colonization of orchid roots by fungal hyphae and the formation of hyphal coils (pelotons) within cortical cells. Pelotons eventually degrade within the cortical cells and their contents are absorbed by the plant. Orchids exploit a range of nutrient strategies from mycoheterotrophy (or parasitism on associated fungi) to mutualism (Rasmussen and Rasmussen 2009; Dearnaley and Cameron 2016). Achlorophyllous orchids rely exclusively on mycoheterotrophy for carbon throughout their lifecycle, while green orchids can produce their own carbohydrates through photosynthesis (Taylor *et al.* 2002). Orchids are commercially important to horticulturalists and are cherished by amateur growers and orchid



clubs. Some orchid species can be grown without fungal partners by supplying germinating dust seeds with a source of simple sugars (Peterson *et al.* 2004).

Ectomycorrhizal associations, which are the focus of this research, occur in only about 2% of vascular plant species but many of those plants are of ecological and commercial importance. Ectomycorrhizas form in a variety of angiosperms and some shrubs and conifer trees (Smith and Read 2008). Many of the trees that associate with ectomycorrhizal fungi are important for logging and paper processing and are of interest to conservationists and foresters. Ectomycorrhizae are characterized by the formation of a Hartig net (or hyphal growth between root cells), a mantle (or fungal hyphae forming a sheath around lateral roots), and extraradical hyphae which grow into surrounding soil. In angiosperms the Hartig net forms only in the root epidermis, whereas in conifers the Hartig net extends between the root cortical cells (Peterson *et al.* 2004). Ectomycorrhizal associations may form with fungal species in the phyla Ascomycota or Basidiomycota and evolved independently over 78 times from saprotrophic fungi between 100 and 200 million years ago (Hibbett *et al.* 2000; Tedersoo and Smith 2013; Kohler *et al.* 2015; Molina and Horton 2015). The same fungal taxa which form ectomycorrhizal associations with most plants may form arbutoid, orchid, or monotropoid mycorrhizae with plants within the families Arbutoideae, Orchidaceae, and Monotropeae respectively.

### *Mycorrhizal Networks and Fungal Forest Ecology*

A forest's mycorrhizal fungal community can be composed of all major types of mycorrhizae and the presence of mycorrhizae depends on the presence of compatible plant and fungal partners. Symbiotic fungi and plants interact on a continuum of specificity; generalist fungi with low specificity will associate with many potential plant partners whereas specialist fungi will

associate with fewer plants (Molina *et al.* 1992; Smith *et al.* 2011; Taylor *et al.* 2002). Whether plants and fungi will enter a mycorrhizal association is likely due to plant-fungus gene interactions established through coevolutionary events (Molina and Horton 2015). Within mixed forest stands mycorrhizal fungi demonstrate some degree of host preference, appearing more commonly on some hosts over others (Hart and Klironomos 2002; Molina and Horton 2015). Plant partners may selectively allocate photosynthetic materials to roots absorbing limiting nutrients (perhaps due to beneficial fungal partnerships) and this allocation of resources may change depending on shifting environmental conditions (Bever *et al.* 2009; Kiers *et al.* 2011). Mycorrhizal fungi fill many different ecosystem niches and a plant may exploit their various functions by associating with multiple fungal partners simultaneously. Meanwhile mycorrhizal fungi may associate with multiple hosts across multiple species (Kennedy *et al.* 2003; Diédhiou *et al.* 2010). Diverse associations between fungi and plants species form interacting linkages within forest ecosystems resulting in common mycelial networks (CMNs) (Trappe and Molina 1982, Simard *et al.* 2012). A schematic of increasingly complex mycorrhizal networks is provided in Horton 2015, Fig. 1.

CMNs may form between various plant and fungal species and fungi forming different mycorrhizal types may link dissimilar plant species. For example, the connection between an autotrophic tree, an ectomycorrhizal fungus, and a mycoheterotrophic plant would represent a simple example of a CMN (Horton 2015). The ability for plants to differentiate between more or less beneficial fungal partners and to allocate resources accordingly may impact the abundance and fruiting responses of those fungi. Changes in environmental conditions such as drought, the introduction of a soil pathogen, or a change in soil nutrient conditions may trigger plants to invest resources to fungal partners differently, and therefore a shift in the environmental conditions in a forest may ultimately lead to shifts in dominant fungal taxa within ecosystems.

# RESPONSES OF ECTOMYCORRHIZAL FUNGI TO CHANGES IN SOIL NUTRIENT CONDITIONS

## *Summary*

Plants form symbiotic relationships belowground with fungal partners. Ectomycorrhizal fungi associate mainly with woody plants and play a significant role in nutrient cycling in temperate forests. Mycorrhizal fungi carry out unique enzymatic processes and aid in plant nutrient uptake. While the importance of mycorrhizal activity on forest nutrient dynamics is acknowledged, various uncertainties remain on the impact of changing nutrient conditions on the function of these symbionts. This section explores the impact of nitrogen and phosphorus additions on ectomycorrhizal fungal symbioses in forests.

## *Introduction*

Anthropogenic nutrient pollutants enter ecosystems through groundwater and atmospheric deposition (Macgregor and Warren 2016). While nitrogen (N) and phosphorus (P) in natural quantities are necessary for ecosystem functions, high levels of the same nutrients can have negative impacts. Increased N and P from human activities such as agriculture and the use of fossil fuels have impacted terrestrial ecosystems by altering soil chemistry and nutrient cycling rates. Forests are exposed to nutrient pollutants mainly through nonpoint source pollution from industries (Davidson 2008). These changes in the nutrient conditions can impact the biota of those systems. One potentially underrated and currently understudied ecological response to changing nutrient dynamics is the presence of mycorrhizal fungi and the composition of mycorrhizal communities.

Many ectomycorrhizal fungi improve N availability to plants. Whereas most non mycorrhizal plants rely on nitrogen fixing bacteria and can only take up N in the forms of

ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ), mycorrhizal fungi uptake N in diverse forms including mineral N. Through unique enzymatic reactions, ectomycorrhizal fungi uptake organic N and deliver it to plant partners in the form of amino acids, ammonium, or nitrate (Plassard *et al.* 1991; 1994).

While gaseous atmospheric nitrogen exists in a form that is inaccessible to most plants alone, inorganic nitrogen fertilizers, such as those transported from agricultural operations, are available to plants without the need for uptake by mycorrhizal symbionts or nitrification by bacterial symbionts (Kytöviita and Arnebrant 2000). Increased anthropogenic deposition of inorganic nitrogen acts as fertilizer and may provide trees with enough nitrogen without mycorrhizal associations (Smith and Read 2008). Resource optimization theory suggests that plants adjust their allocation of carbon to acquire limiting resources (Bloom *et al.* 1985). Plants can access limiting nutrients through their mycorrhizal associations (Smith and Read 2008). Changing nutrient conditions may negate certain mycorrhizal functions and have been shown to negatively impact the diversity and abundance of some ectomycorrhizal fungi (Peter *et al.* 2001; Lilleskov *et al.* 2002). Initial evidence summarized by Arnolds (1991) pointed to a decrease in the diversity and abundance of ectomycorrhizal fungal sporocarps throughout Europe correlated with increased atmospheric nitrogen. This hypothesized effect of increased nitrogen deposition has been confirmed through subsequent nitrogen fertilizations experiments (Wallenda and Kottke 1998; Lilleskov *et al.* 2001b; Lilleskov *et al.* 2002).

While previous work has informed researchers of ectomycorrhizal responses to nitrogen, the functional relationship of nutrient availability and carbon allocation to specific mycorrhizal taxa remains largely elusive. Little is known about how certain ectomycorrhizal species might

respond to changing limiting nutrients (Lilleskov and Bruns 2001). Some species of mycorrhizal fungi have been shown to be more distinctly impacted by N deposition than others (Peter *et al.* 2001; Lilleskov *et al.* 2011). While ectomycorrhizal fungal symbionts have been noted for their ability to uptake organic and inorganic forms of N for host plants, different species fulfill different ecological niches beyond their ability to acquire nutrients (Smith and Read 2008). The loss of nutrient mining taxa may impact plants' access to other important fungal ecosystem services as well, including increased drought resistance (Pickles and Simard 2017) and soil pathogen protection (Shelkle and Peterson 1997). By examining the responses of different taxa to fertilization, researchers can observe whether responses are reflective of fungal functional traits (Lilleskov 2010).

#### *Nutrient Limitation and Pollution in Hardwood Ecosystems*

Plant productivity is constrained by limiting resources. Terrestrial ecosystems are commonly expected to be limited by either nitrogen or phosphorus (Elser *et al.* 2007; Davidson 2008). Broadly, variation between nitrogen and phosphorus limitations in forest ecosystems can be correlated with soil age (Walker and Syers 1976; Vitousek and Farrington 1997). Accessible soil nitrogen is derived largely from the decomposition of organic materials, therefore, the net primary productivity and net ecosystem productivity of forests with relatively young soils is expected to be nitrogen limited (Stevens and Walker 1970; Finzi 2009). Phosphorus, in contrast, is largely mined from bedrock apatite by fine roots and hyphae (Blum *et al.* 2002). Mature ecosystems are expected to reach a condition of phosphorus limitation because mined mineral phosphorus cannot be recharged through nutrient cycling the way that nitrogen can (Walker and Syers 1976; Vitousek *et al.* 2010).

Contrary to previously understood conditions of single element limitation, model simulations based on resources optimization theory suggest that ecosystems could be colimited by both nitrogen and phosphorus (Bloom *et al.* 1985, Chapin *et al.* 1986). This concept is also referred to as the "functional equilibrium hypothesis" (Rastetter *et al.* 1997a). When faced with a resource limitation, organisms respond by allocating greater investment towards acquiring that resource (Tilman 1982; Bloom *et al.* 1985; Chapin 1991). Consequently, increased allocation of carbon to roots, as indicated by a decreased production of fine roots as well as mycorrhizal root tips has been correlated to sites where essential nutrients are limiting (Wallenda and Kottke 1998; Bae *et al.* 2015), but this response may differ according to tree species and mycorrhizal associations (Shan *et al.* 2018). In alternative ecosystem models by Menge *et al.* (2012), various environmental histories determine whether a system might converge on nitrogen or phosphorus limitation. At all modeled successional timescales, symbiotic nitrogen fixation was found to have the capacity to overcome N limitation, suggesting that nutrient limitation depends on the activity of soil symbionts (Menge *et al.* 2012).

### *Nitrogen Dynamics and Ectomycorrhizal Fungi*

Ectomycorrhizal fungi can acquire nitrogen in forms not otherwise available to plants. There is variation, however, regarding the role that different fungal taxa have in acquiring specific forms of nitrogen (Plassard *et al.* 1991, 1994; Lilleskov and Bruns 2001; Lilleskov *et al.* 2001; Lilleskov *et al.* 2002). When grown aseptically in culture and exposed to various forms of N, ectomycorrhizal taxa were found to uptake ammonium preferentially, but this uptake was regulated by the presence of organic nitrogen sources (Read *et al.* 2004). Enzymes necessary for ammonium uptake have been identified in *Paxillus involutus* and the mechanisms for molecular transfer of

various forms of N are slowly becoming clearer (Javelle *et al.* 2003a; Lilleskov *et al.* 2011). Research on gene expression in various ectomycorrhizal fungal species as well as isotopic tracking have indicated specific patterns of nitrogen uptake across taxa (Lilleskov *et al.* 2002; Kytöviita and Arnebrant 1999). Further research into the exact mechanisms for nitrogen uptake by ectomycorrhizal fungi have elucidated some patterns in nitrogen relations across taxa to predict which taxa (including *Cortinarius*, *Boletus*, *Suillus*, *Piloderma*, and *Tricholoma*) are disproportionately impacted by anthropogenic nitrogen additions (Lilleskov *et al.* 2011). A loss of certain EMF taxa within the soil community following N addition may suggest that those fungal types are most responsible for acquiring organic N and transferring it to plants.

Over an anthropogenic N deposition gradient in Kenai, Alaska, Lilleskov *et al.* (2001b) observed a loss of mycorrhizal taxa and a shift in dominant species under high N inputs. Nitrogen deposition in this study decreased as distance from an existing ammonium production facility increased. Notably, at the six highest nitrogen sites, researchers encountered only 14 species, where they encountered 144 species at the six lowest N sites. While sporocarps in this study were sampled intensely at 2-3 week intervals throughout three seasons, researchers indicate the necessity of coordinated belowground sampling (Lilleskov *et al.* 2001b).

Peter *et al.* (2001) combined above and belowground sampling efforts to better understand community level effects of short-term nitrogen addition (sampling occurred both before and after two years of fertilization). The results of this sampling showed above and belowground responses to nitrogen additions amongst EM fungi but no difference in saprophytic fungi (Peter *et al.* 2001). Belowground responses of EM fungi to N deposition were less immediate and less drastic than aboveground responses, indicating that either; 1. Belowground community composition responses

occur less immediately after N addition than the response of sporocarp productivity, or 2. N addition does not impact belowground fungal communities but does impact allocation of resources to fruit body production (Peter *et al.* 2001). Evidence for the former hypothesis has been supported by later experiments that relied on soil sampling and analyses of root colonization by ectomycorrhizal hyphae to quantify nutrient effects (Horton and Bruns 2001). In ecosystems that are no longer N limited due to heavy deposition of inorganic N, plants do not rely as heavily on fungal associations to acquire N and the number of mycorrhizal root tips on their root systems has been shown to decrease (Meyer 1988; Treseder 2004). This points to the potential for fertilization to impact the composition of soil fungal communities as species that specialize in N uptake may be starved of carbon from their plant partners to save resources for more beneficial partnerships (Arnolds 1991; Bever *et al.* 2009).

Vitousek and Howarth (1991) suggested that nitrogen limitation across a wide range of ecosystems would select disproportionately for nitrogen fixing plants. Further, they suppose that nitrogen limitation should eventually be alleviated due to the activity of N fixing plants (Vitousek and Howarth 1991). Nitrogen could remain a primary limiting nutrient, however, because of the high cost of photosynthetic energy needed for a plant to support symbiotic nitrogen fixing bacteria (Gutschick 1981; Vitousek and Howarth 1991). N-fixing plants, such as Alders, may form highly specific associations with very few EM fungi (Horton *et al.* 2013). Associations with mycorrhizal symbionts may serve as a more energy efficient life strategy for some plants to acquire nitrogen in ecosystems where it limits productivity (Kucey and Paul 1981; Hobbie *et al.* 2000). Hobbie *et al.* (1998) concluded that N cycles more quickly in early successional stands dominated by the EM and N-fixing tree *Alnus sinuata* than in late successional stands dominated by the EM tree *Picea*



*sitchensis*. Horton *et al.* (2013) hypothesized that the EM fungal species associated with N-fixing plants may be important in acquiring P to facilitate N-fixation by N fixing bacteria.

Symbiosis with ectomycorrhizal fungal partners can be more or less metabolically expensive for plant hosts in different ecological contexts (Linder and Axelsson, 1982; Johnson *et al.* 1997). Associations between mutualistic fungi and their plant partners exist largely because each organism is limited by a resource that the other, or others, provide(s) (Smith and Read, 2008). Changes in the nutrient conditions of an ecosystem away from nitrogen limitation have been shown to negate a plant's needs for nitrogen acquired by mycorrhizal fungi (Arnolds, 1991; Wallenda and Kottke, 1998; Lilleskov *et al.* 2001). While EM fungal abundance and species richness have been shown to decline both above and belowground, some genera of fungi have been shown to be more distinctly impacted by nitrogen deposition than others (Peter, *et al.* 2001; Lilleskov *et al.* 2002a; Lilleskov *et al.* 2002b; Hobbie and Agerer 2010). The relative positive, negative, or neutral nitrogen responses common amongst certain taxa apparently follow trends of shared ecosystem functions of those fungi (Hobbie and Agerer 2010; Hobbie and Hogberg 2012; Lilleskov *et al.* 2011). Different fungal species display distinct functional traits allowing them to fill different ecological niches (Smith and Read, 2008) and EM fungi may respond to N deposition differently based on functional species niche. Hyphal exploration type, carbon demand from hosts, nutrient mining acquisition, and hydrophobicity are functional traits that may influence how well adapted a given fungal species is to acquire organic N and this adaptation likely impacts how sensitive that species is to nitrogen deposition (Hobbie and Agerer 2010).

Lilleskov *et al.* (2002a) indicates low N and high N taxa as 'nitrophobic' and 'nitrophilic', respectively. These taxa have been shown to respond differently under increased N. For example,

EM fungal types associated with conifers have been correlated with greater sensitivity to N deposition than those that associate with broadleaf trees (Arnolds 1991; Cox *et al.* 2010; van der Linde *et al.* 2018). Further, genera that require greater carbon allocation from their host plants may be impacted more significantly by N deposition. Fungi with a larger belowground thallus, namely those that produce the medium-distance fringe exploration type hyphae such as species of *Tricholoma*, *Cortinarius*, and *Piloderma*, have displayed greater reductions in abundance following N addition (Agerer 2001; Agerer 2006; Hobbie and Agerer 2010). This correlation is likely due to the role of those fungi as miners of limiting nutrients for their plant hosts. Mycorrhizas with hydrophilic extraradical hyphae often lack the ability to access soluble nitrogen and are less impacted by high nitrogen conditions (Hobbie and Agerer 2010). Mycorrhizas with hydrophobic rhizomorphs, alternatively, acquire soluble nitrogen for their hosts and are better suited for nitrogen limited environments (Hobbie and Agerer 2010). Unpublished work from Lilleskov *et al.* indicate *Thelephora* and *Laccaria* as nitrophilic genera whereas *Cortinarius*, *Tricholoma*, *Piloderma*, *Bankeraceae*, and *Suillus* are consistently indicated as nitrophobic. The capacity for EM fungal taxa to mine and transport labile or complex organic N depends on the production of a suite of N mobilizing enzymes (Hobbie and Agerer 2010; Lilleskov *et al.* 2011). Fungi with hydrophobic rhizomorphs especially in medium to long-distance fringe exploration types seems to correspond consistently to the sensitivity of those taxa to N deposition (Lilleskov *et al.* 2011). Understanding the functional traits of a given fungal species may be helpful in predicting how that species responds to changes in soil nutrient conditions. More research is required to fully understand the enzymatic capabilities of different fungal species to acquire N in different forms.

Forest productivity in the northeastern United States is expected to be nitrogen limited but in areas experiencing long term atmospheric nitrogen deposition forests may approach phosphorus limitation (Almeida *et al.* 2018). In stands in New Hampshire treated with N and P in a factorial design aboveground plant biomass, as indicated by relative basal area increase (RBAI), in both mid-aged and mature stands responded positively to P fertilization (Goswami *et al.* 2018). This response suggests that aboveground productivity in these stands is P limited. In plots without added P fine roots foraged for apatite derived P in in-growth cores, further supporting that these stands are P limited (Shan 2020). Fine root biomass increased in N addition plots suggesting that while aboveground biomass in BEF is P limited, belowground root biomass is N limited (Shan 2020). Changes in microbial respiration, and fungal and microbial abundance, were driven by changes in N (Shan *et al.* 2018). In soils dominated by *Acer rubrum* (red maple), an AM associated tree, N additions increased microbial respiration and decreased soil fungal abundance (Shan *et al.* 2018). When soils were dominated by *Betula alleghaniensis* (yellow birch), an EM associated tree, soil respiration decreased along with fungal biomass suggesting that belowground nutrient effects strongly depend on whether dominant trees associate with AM or EM fungi (Shan *et al.* 2018).

In a boreal forest in southwest Sweden where N and P were also added in a factorial design, the same aboveground response was recorded; aboveground plant biomass increased in P treated plots (Almeida *et al.* 2018). In this study EM fungal biomass reduced only when N and P were added together (Almeida *et al.* 2018). Though it has been reported that extraradical biomass of ectomycorrhizal fungi proliferate in low P conditions (Wallander 1995; Rosenstock 2009), Almeida *et al.* (2018) found an increase in fungal biomass when P limitation was alleviated via

apatite additions. P addition has been shown to stimulate fungal biomass (Hagerberg *et al.* 2003; Almeida *et al.* 2018) but this effect disappeared when P was added in a stand that was not P limited (Wallander and Thelin 2008).

Phosphorus is available to plants largely in the form of apatite. Apatite mining roots and hyphae acquire P and other nutrients bound in bedrock but the role of P uptake for plants is largely attributed to AM fungi rather than EM fungi (Stevens and Walker 1970; Walker and Syers 1976; Jakobsen 1995; Jakobsen *et al.* 2005a). Significant and consistent N effects on EM fungi have been recorded but less information is available on the effect of P on mycorrhizal associations and on specific EM fungi. An increase in fungal biomass following apatite amendment was supported by Berner *et al.* (2011) but despite biomass increases no change in fungal community structure was reported. As familiarity with the functional niches of specific taxa increases, inferences may be made and tested about how different species interact with phosphorus. Similarly to mycorrhizal interactions with changing soil nitrogen levels, we may begin to see distinctions between ‘phosphophilic’ and ‘phosphophobic’ genera in the case of changing soil phosphorus levels.

### *Conclusion*

Ectomycorrhizal fungi interact with their environments and respond to changing environmental conditions. Under changing environmental conditions, the nature of symbioses between ectomycorrhizal fungi and their plant host may undergo changes as well. Because plants form relationships with fungal partners to acquire limiting resources, changes in nutrient limitations are particularly impactful on fungal community composition. Different species of mycorrhizal fungi have different roles within ecosystems. These functional niches seem to correlate with loss of diversity when the roles of fungi important in nutrient acquisition are negated

by increased resource availability. While researchers have progressed to a greater understanding of specific interactions between various ectomycorrhizal taxa and nitrogen deposition, interaction between those taxa and phosphorus deposition are yet to be uncovered. Chapter 2 provides results of a rigorous sporocarp survey in plots treated with nitrogen and phosphorus in a fully replicated factorial design. Results will address previously unclear relationships between N and P additions and the reproductive responses of many fungal taxa.

## THESIS LAYOUT

The main body of my thesis is composed of three chapters including this literature review chapter, a manuscript style chapter, and a concluding chapter. Chapter 1 introduces the history of the study of symbiosis, fungal and mycorrhizal ecology, and the current literature on EMF responses to N and P additions.

Chapter 2 details my field and laboratory work and presents the results of my 2018 sporocarp survey in plots treated with N and P in a factorial design. I collected ectomycorrhizal fruit bodies, grouped them into morphospecies, confirmed those morphospecies groups using molecular techniques, and carried out analyses on sporocarp abundance, biomass, and community assemblage.

I briefly conclude my work in chapter 3, connecting my findings to current literature from chapter 1. Here I consider the implications of changing soil nutrient conditions for mycorrhizal ecology. Sporocarps respond more drastically and quickly to changing environmental conditions than mycorrhizal roots and may be useful for observing ecosystem changes on a shorter timescale. Finally, I consider paths which my own project could take if ever time and money allowed.

## **Chapter 2: Fruiting response of ectomycorrhizal fungi to nutrient additions in Bartlett Experimental Forest, New Hampshire**

### **ABSTRACT**

Ectomycorrhizal (EM) associations are fundamental to normal forest ecosystem functions in stands dominated by EM trees, but EM fungi may be sensitive to soil nutrient additions. This research investigates fruiting responses of ectomycorrhizal fungi to nutrient additions in a project on Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) in which nitrogen (N) and phosphorus (P) have been added annually since 2011. To quantify the response of N and P additions on ectomycorrhizal fungal (EMF) fruiting, EMF sporocarps were collected and quantified five times from July – October 2018, in six stands across two successional stages: mid-aged (harvested between 1970-1979) and mature (harvested between 1880-1890). Morphological types (morphospecies) were confirmed using the fungal barcode (nrITS region). Data were analyzed using linear mixed models and multivariate community ordination. Sporocarp abundance and species richness was suppressed in N addition plots. Sporocarp community composition, described by ordination, responded to N and P additions, and changed over the season. Indicator species were observed in control and P addition plots. While mycorrhizal fungi are known to respond to N fertilization, this work is among the first to observe a sporocarp community response to P fertilization. Measuring changes in sporocarp production provides information on the reproductive output of fruiting genera, which was used as a proxy to observe how those genera respond to changes in nutrient availability.

**Key Words:** ectomycorrhizal fungi, community ecology, forest ecology, nitrogen, phosphorus, nutrient limitation

## INTRODUCTION

Across all groups of life, the earth is losing biodiversity at an alarming rate (Kim and Byrne 2006). Due to the ephemeral nature of sporocarps and the difficulty of identifying most fungi, fungal species richness is often underrepresented in biodiversity inventories. Mycorrhizal fungi fill a range of ecosystem niches and form associations with most plant families (Smith and Read 2008; van der Heijden *et al.* 2015). Due to the nearly ubiquitous need for mycorrhizal associations by plants, the presence and diversity of these fungi is fundamental to ecosystem functioning, but we are yet to understand the extent of functional divergence between different species of mycorrhizal fungi (Smith and Read 2008; Hobbie and Agerer 2010). The biodiversity of mycorrhizal fungi is sensitive to changes in ecosystem conditions but the responses of many fungi to specific disturbances and changes remains elusive (Lilleskov *et al.* 2011). Without a clear understanding of fungal species richness and functional diversity we have little power in measuring the loss of fungal species biodiversity and predicting how this loss will impact ecosystems locally and globally. This study relies on sporocarp collection and identification to understand the impact of nutrient additions on the fruiting patterns of ectomycorrhizal fungi.

Nitrogen (N) and phosphorus (P) are the most common limiting nutrients in terrestrial ecosystems (Vitousek *et al.* 2010) and added nutrients can impact normal ecosystem functions. Plants allocate a large portion of available carbon belowground to acquire limiting soil nutrients, largely through associations with mycorrhizal fungi (Ericsson *et al.* 1996; Smith and Read 2008). Increased nutrient availability significantly impacts the fertility of forest soils, affecting tree carbon allocation (Janssens *et al.* 2010). Plant partners may selectively allocate photosynthetic materials to roots that are absorbing limiting nutrients (perhaps due to beneficial fungal partnerships) and this allocation of resources may change depending on shifting environmental conditions (Bever *et*

*al.* 2009; Kiers *et al.* 2011). In response to increased nutrient availability plants may decrease their allocation of carbon belowground, as indicated by a decreased production of fine roots and mycorrhizal root tips (Ritter and Tölle 1978; Ericsson 1995; Bae *et al.* 2015). Decreased allocation of carbon to roots by host trees affects ectomycorrhizal fungal (EMF) biomass above and belowground as well as overall community assemblage (Arnolds 1991; Peter *et al.* 2001; Lilleskov *et al.* 2002a.; Lilleskov *et al.* 2002b).

Anthropogenic nutrient deposition may provide trees with enough nutrients, thus diminishing the need for some mycorrhizal associations (Lilleskov *et al.* 2001). The most established example of this is the impact of inorganic N deposition on mycorrhizal abundance and fungal species richness (Arnolds 1991; Lilleskov and Bruns 2001; Lilleskov *et al.* 2002a; Lilleskov *et al.* 2011; Treseder 2004; Wallenda and Kottke 1998). If plants do not need fungal associations to acquire N, they stop providing carbon to host roots, and studies have shown EMF important for N uptake under N-limited conditions may decline in areas experiencing high N deposition (Arnolds 1991). The impact of P additions on ectomycorrhizal productivity is less clear. P is mined from bedrock by fungal hyphae and plant roots, but P uptake tends to be attributed to arbuscular mycorrhizal (AM) fungi rather than EMF (Stevens and Walker 1970; Walker and Syers 1976; Jakobsen 1995; Jakobsen *et al.* 2005a). While belowground fungal biomass may increase under elevated P (Hagerberg *et al.* 2003), that effect disappears when P is added in a stand that is not P limited (Wallander and Thelin 2008). Other studies have indicated high EMF production in low P environments, suggesting that some EMF likely play an important role in P mining and acquisition (Wallander and Nylund 1992; Rosenstock *et al.* 2016).



The roots of a single tree may be colonized by tens of genets across hundreds of fungal species, many of which are equipped to perform different ecosystem services (Braham *et al.* 2011; Horton 2015). In addition to improving nutrient and water accessibility for plants, EMF also reduce root herbivory and protect plants from soil pathogens (Hobbie and Agerer 2010). Therefore, a change in a plant's reliance on EMF for nutrient uptake may impact other functional benefits of plant-fungal symbioses for trees and ecosystems (Hobbie and Agerer 2010).

Reduced belowground allocation of carbon following N additions may select for 'nitrophilic' fungal taxa, or those well adapted to high N environments (Lilleskov *et al.* 2001). 'Nitrophilic' fungal taxa are those that likely do not play a strong role in acquiring soil N. There is evidence that many low biomass mycorrhizal fungi with contact, short-, and medium-distance smooth exploration type hyphae persist in high N systems whereas many high biomass taxa with medium-distance fringe, medium-distance mat and long-distance exploration types decrease in species diversity and abundance under these high N conditions (Lilleskov *et al.* 2001; Hasselquist and Högberg 2014). Species within the genera *Tricholoma*, *Cortinarius*, and *Piloderma*, have displayed consistent reductions in abundance and species richness following N addition, and can be considered 'nitrophobic' taxa (Agerer 2001; Agerer 2006; Hobbie and Agerer 2010). This response is possibly because these taxa are adapted to mine for organic N under N-limited conditions (Lilleskov *et al.* 2011). The length of extraradical hyphae and the production of a suite of N mobilizing enzymes seem to be the most important determining factors to predict whether a species will respond negatively to N deposition (Lilleskov *et al.* 2011).

In systems where N has been added but where P is limiting, trees may allocate carbon belowground to roots supporting P mining hyphae, but little is known about which EMF are

important for P acquisition. P limitation likely selects for different fungal taxa than N limitation due to differences in competitive fungal traits under different environmental conditions. With further investigation we may begin to understand which groups of EM fungi are ‘phosphophilic’, or persistent following P depositions and which are ‘phosphophobic’, or sensitive to P deposition.

The MELNHE (Multiple Element Limitation in Northern Hardwood Ecosystems) project has added N and P in a fully replicated factorial design for nine years. These added nutrients model forest systems that have been exposed to nutrient pollution. In mid-aged and mature stands in BEF aboveground forest productivity, as indicated by per- tree annual relative basal area increment (RBAI), responded to P fertilization (Goswami *et al.* 2018). Further, RBAI in P addition plots was greater among EM associated trees than AM associated trees (Goswami *et al.* 2018). Increased aboveground productivity following P additions suggests that these stands are P limited.

Fine root growth in the same stands responded positively to N additions suggesting that while P limits aboveground plant productivity, N may limit belowground plant productivity (Shan 2020). Rhizosphere effects, including microbial activity and microbial and fungal abundance, also responded to N additions but the direction of this effect differed depending on whether rhizosphere soils were collected from trees associated with arbuscular mycorrhizal or ectomycorrhizal fungi (Shan *et al.* 2018). In AM associated red maple soils, N additions increased microbial respiration and decreased soil fungal abundance as quantified by qPCR (Shan *et al.* 2018). In EM associated yellow birch soils, microbial respiration decreased along with belowground fungal abundance in response to N addition (Shan *et al.* 2018). Differing responses above and belowground may support theories of colimitation between N and P.

To examine the response of EMF fruiting to N and P additions I utilized three mid-aged and three mature MELNHE stands. I analyzed the impact of N, P, and N+P additions on aboveground EMF biomass, sporocarp abundance, species richness, and community composition. EMF species are functionally divergent in their nutrient uptake and exchange capacities with trees, such that nutrient additions influences EMF sporocarp productivity.

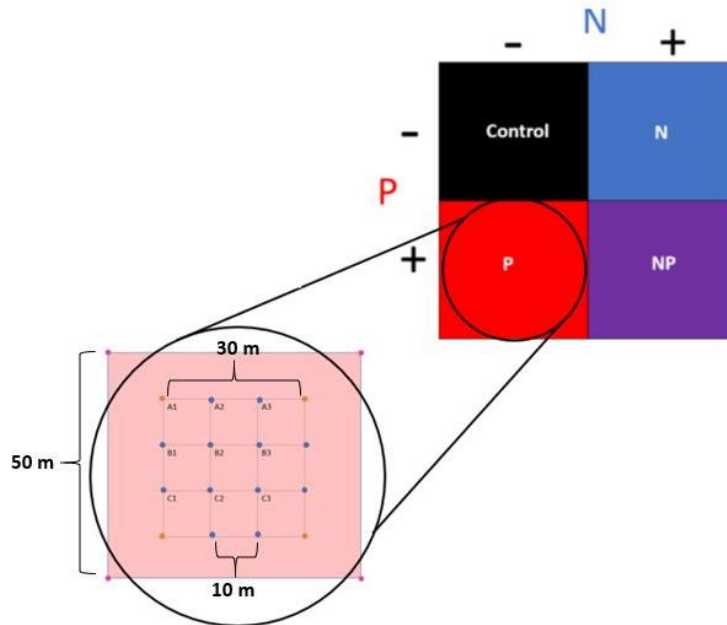
My hypotheses were:

1. EMF sporocarp community composition will change according to nutrient conditions.  
Community composition in N, P, and N+P addition plots will be dissimilar from control plots.
2. EMF sporocarp abundance, species richness, and biomass will be suppressed in N addition plots.
3. EMF sporocarp abundance, species richness, and biomass will not respond significantly to P additions.

## MATERIALS AND METHODS

**Site Description:** The Bartlett Experimental Forest (BEF) (lat. 44°2'39" N, long. 71°9'56" W) is located in the White Mountains, New Hampshire, USA. The region experiences warm summers with high temperatures often above 32° C and average July temperatures of 19° C. Winter temperatures fall below 0° C with average January temperatures of -9° C. Average annual precipitation is 1,270 mm distributed throughout the year (USFS Northern Research Station). Soils are spodosols which developed on glacial till derived from granite and gneiss (USFS Northern Research Station).

Six stands in BEF were harvested at varied times resulting in mid-aged (30-35 years since harvest), and mature (> 100 years since harvest) stands (Table 1). Each stand included four 30x30 m plots receiving N and P additions in a factorial design (+N, +P, +N and P, and control). An additional 10x10 m treated buffer surrounded the collection area of each plot. Each stand contained a control plot (C), as well as plots fertilized yearly since 2011 with N (30 kg N ha<sup>-1</sup> yr<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>), P (10 kg P ha<sup>-1</sup> yr<sup>-1</sup> as NaH<sub>2</sub>PO<sub>4</sub>) and both N and P at the same rates. A schematic of the factorial design and the plot layout are provided in Figure 1 below.



**Figure 1:** Schematic representation of factorial design in each stand. Each of six sampled stands is divided into four plots receiving N, P, both N and P, or no added nutrients (control). Plots were sampled excluding the buffer area.

Stand ages were based on the time from harvest until fertilization began in 2011. Forest stand composition varied with Betulaceae, Fagaceae, and Sapindaceae dominating most stands (Table 3, Figure 2). Of the ectomycorrhizal tree species *Fagus grandifolia* (BE) and *Betula alleghaniensis* (YB) were dominant in mature stands whereas *F. grandifolia*, *B. papyrifera*, *B. populifolia* (WB), and *Populus grandidentata* (BA) were more prevalent in mid-aged stands (Table

2, Table 3, Figure 2). Details of the MELNHE stand inventories can be found in Goswami *et al.* (2018) and are summarized here in Tables 2 and 3, and Figure 2.

**Table 1:** Site characteristics for all sampled stands in Bartlett Experimental Forest NH. All MELNHE stands in BEF were named starting with a ‘C’, which stands for clear-cut, and then numerically. C4-C6 are mid-aged stands, and C7-C9 are mature stands.

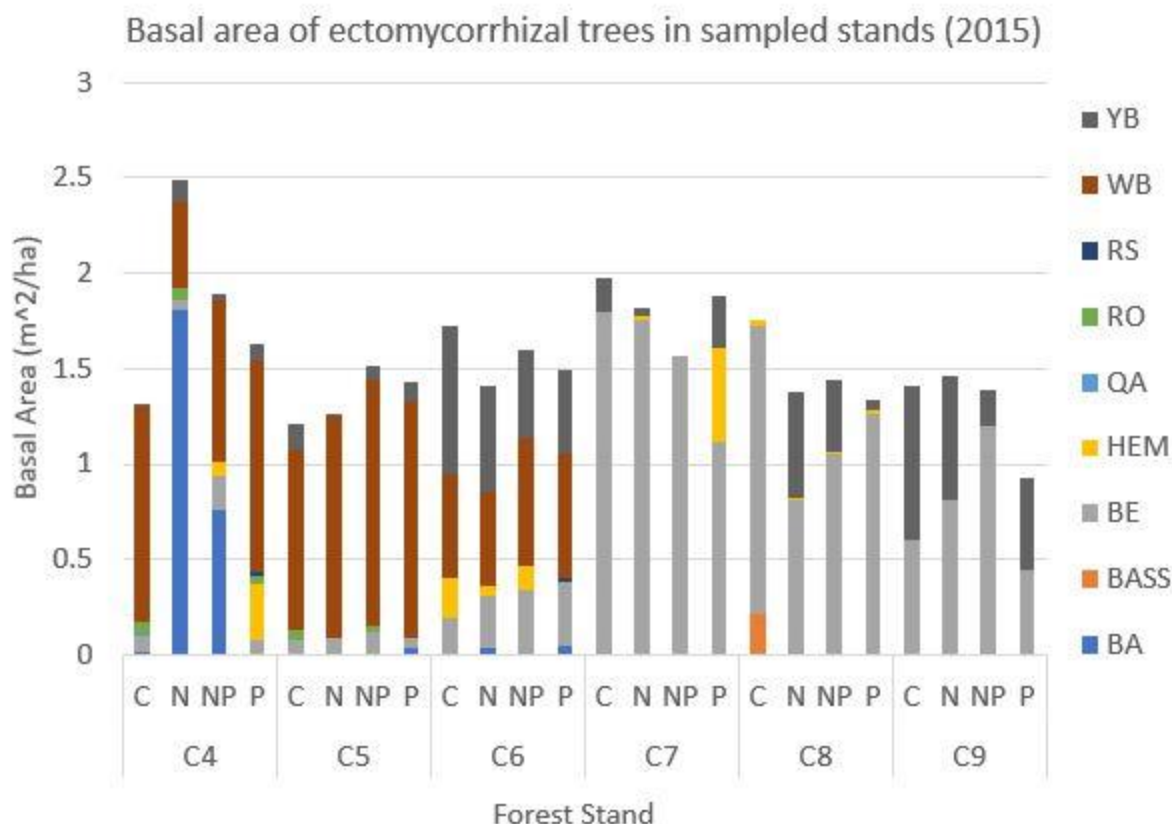
Stand	Forest age	Year clear cut	Elevation (ft)	Aspect	Slope (%)
C4	mid-age	1979	410	Northeast	20–25
C5	mid-age	1976	550	Northwest	20-30
C6	mid-age	1975	460	North-northwest	13-20
C7	mature	1890	440	East-northeast	5-10
C8	mature	1883	330	Northeast	5-35
C9	mature	1890	440	Northeast	10-35

**Table 2:** Vegetation data species codes along with mycorrhizal status as ectomycorrhizal (EM) or arbuscular mycorrhiza (AM) of inventoried trees in BEF.

ID	Common name(s)	Scientific name(s)	Fungal Ecology
ASH	White Ash or Mountain Ash	<i>Fraxinus americana</i>	AM
QA	Quaking Aspen	<i>Populus tremuloides</i>	EM
BA	Bigtooth Aspen	<i>Populus grandidentata</i>	EM
BASS	Basswood	<i>Tilia americana</i>	EM
BE	American Beech	<i>Fagus grandifolia</i>	EM
FIR	Balsam Fir	<i>Abies balsamea</i>	EM
HEM	Eastern Hemlock	<i>Tsuga canadensis</i>	EM
MM	Mountain Maple	<i>Acer spicatum</i>	AM
PC	Pin Cherry	<i>Prunus pensylvanica</i>	AM
RM	Red Maple	<i>Acer rubrum</i>	AM
RO	Northern Red Oak	<i>Quercus rubra</i>	EM
RS	Red Spruce	<i>Picea rubens</i>	EM
SM	Sugar Maple	<i>Acer saccharum</i>	AM
WB	Paper (White) Birch or Gray Birch	<i>Betula papyrifera</i> or <i>B. populifolia</i>	EM
YB	Yellow Birch	<i>Betula alleghaniensis</i>	EM

**Table 3:** Dominant tree species and families in each sampled stand BEF and mycorrhizal associations

<b>Stand</b>	<b>Dominant Tree Species <math>\geq 10\text{cm}</math> DBH</b>	<b>Tree Family</b>	<b>Proportion of Trees</b>	<b>Mycorrhizal association</b>	<b>Dominant Fungal Partner in Stand</b>
<b>C4</b>	BA	Salicaceae	20%	EM	<b>77% EM</b>
	BE	Fagaceae	7%	EM	
	PC	Rosaceae	12%	AM	
	RM	Sapindaceae	8%	AM	
	WB	Betulaceae	44%	EM	
	YB	Betulaceae	6%	EM	
<b>C5</b>	BE	Fagaceae	6%	EM	<b>80% EM</b>
	PC	Rosaceae	11%	AM	
	RM	Sapindaceae	6%	AM	
	WB	Betulaceae	69%	EM	
	YB	Betulaceae	5%	EM	
<b>C6</b>	ASH	Oleaceae	2%	AM	<b>58% EM</b>
	BE	Fagaceae	15%	EM	
	HEM	Pinaceae	2%	EM	
	PC	Rosaceae	16%	AM	
	RM	Sapindaceae	19%	AM	
	SM	Sapindaceae	2%	AM	
	STM	Sapindaceae	2%	AM	
	WB	Betulaceae	19%	EM	
	YB	Betulaceae	22%	EM	
<b>C7</b>	BE	Fagaceae	67%	EM	<b>73% EM</b>
	SM	Sapindaceae	19%	AM	
	YB	Betulaceae	6%	EM	
<b>C8</b>	BE	Fagaceae	62%	EM	<b>70%EM</b>
	SM	Sapindaceae	24%	AM	
	WB	Betulaceae	8%	EM	
<b>C9</b>	BE	Fagaceae	44%	EM	<b>62% EM</b>
	SM	Sapindaceae	37%	AM	
	YB	Betulaceae	18%	EM	



**Figure 2:** Basal area of ectomycorrhizal tree species in C4 - C9 treatment plots based on 2015 forest inventory of trees  $\geq 10$  cm DBH in Bartlett Experimental Forest. Species codes provided in Table 2.

**Sampling Methods:** Over the course of the 2018 fungal growing season the three mid-aged and three mature fertilized stands in the BEF were sampled five times for ectomycorrhizal sporocarps. Sampling efforts ranged from late July through mid-October. The 30x30 m sampling area was divided into nine 10x10 m subplots and each subplot was sampled using a three-and-a-half-minute timed wander to ensure that the sampling effort was consistent across the whole plot. Sporocarps were counted, photographed, and sorted into morphospecies groups based on macroscopic and microscopic morphological features. Each collected sporocarp was dried on a food dehydrator, given a unique label, and stored in labeled plastic bags with desiccant for subsequent molecular work and to serve as vouchers. Sporocarps collected from the center subplot of each plot were weighed to provide data on aboveground fungal biomass.

**Table 4:** Trip dates and weather conditions for each of five sampling efforts

Trip	Dates (2018)	Average weekly temperature (C)	Average weekly Precipitation (In)
1	July 27-29	21.31° C	0.30 in
2	August 13-15	21.37 ° C	0.14 in
3	August 30-September 2	22.34 ° C	0 in
4	September 24 - September 27	13.62 ° C	0.29 in
5	October 12 - October 15	8.37 ° C	0.05 in

Note: data were collected from Weather Underground (<https://www.wunderground.com/>)

**Identification of Ectomycorrhizal Sporocarps by Morphology:** Sporocarps collected from the field were initially sorted into species concepts based on macroscopic and microscopic morphology (morphospecies). These preliminary morphospecies identifications were based largely on fresh specimens. Dried specimens and photos were occasionally revisited to record additional information. The structure of the specimens hymenial layer and the color of the specimen's spores were important initial observations. Gill morphology, the presence or structure of a stipe, staining reactions to KOH, ammonium, or ferrous sulfate, as well as the presence or remnants of a volva, cortina, or universal veil were also considered. Microscopic features considered included the shape, size, and ornamentation of spores and occasionally the presence of notable cystidia. Dichotomous and picture keys were used to compare specimens to recorded species descriptions (Arora 1986; Lincoff 1997; Baroni 2017; Bessette *et al.* 2016; Bassette *et al.* 2010). The ecology of each morphospecies was confirmed using the FUNGuild database (Nguyen *et al.* 2018).

**Molecular Methods:** DNA was extracted from sporocarp tissue of representatives from each morphospecies using the CTAB method and extracted DNA was used to mix 1:100 dilutions in molecular grade water (Gardes and Bruns 1993). The nuclear ribosomal internal transcribed spacer (nrITS) region was amplified by polymerase chain reaction (PCR) using ITS1-F for the



forward primer (Gardes and Bruns 1993) and ITS4 or ITS4-B for reverse primers following Gardes and Bruns (1993) and White *et al.* (2014). Samples were run through a 3% agarose gel in 1XTBE buffer, stained using ethidium bromide, rinsed in tap water, and imaged using a Gel Doc EZ System (Bio-rad, Hercules, CA, USA).

Successfully amplified samples were digested in 15 µl reactions using the restriction enzymes *Hinf*I and *Dpn*II following the manufacturer's protocols (New England Biolabs, Ipswich, NH). Again, these reactions were run on a 3% agarose gel using the methods described above. ITS samples with the same restriction fragment length polymorphism (RFLP) patterns were grouped and considered operational taxonomic units (OTU). Representatives of each unique combination of morphospecies and RFLP pattern were selected to be reamplified for sequencing. Unique RFLP patterns were not considered OTUs to avoid errors due to shared RFLP patterns across two or more species (Avis *et al.* 2006; Dickie and FitzJohn 2007). Samples were reamplified using the same primers (ITS1-F and ITS 4 or ITS4-b). Gel electrophoresis was used to confirm successful amplification and these PCR products were cleaned using QIAquick PCR purification kit columns (Qiagen, Valencia, CA, USA). DNA concentration was quantified using a ND-1000 NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA concentrations of the samples were adjusted to 20-40 ng/µl and sent to Eurofins (Louisville, KY) for DNA sequencing using the primer ITS-1F.

Sequences returned from Eurofins were visually analyzed and edited using FinchTV version 1.4.0. and BioEdit version 7.2.1. Cutadapt 1.8 (Martin 2011) was used to trim sequences. Next, sequences were clustered into denovo operational taxonomic units (OTUs) with the QIIME 2 VSEARCH plug-in, by first clustering at 98.5%, then clustering the resulting representative

sequences for 98.5% OTUs at 97% similarity (Taylor *et al.* 2000; Schoch *et al.* 2012). The QIIME 2 feature-classifier plug-in (Bokulich *et al.* 2018) was used to assign taxonomy to representative sequences of 97% clusters using the naive Bayes classifier (Fabian *et al.* 2011). Taxonomic classification used the QIIME formatted UNITE dynamic species hypothesis dataset (version 8.0, released 02.02.2019; Kõljalg *et al.* 2013). Taxonomy was also assigned individually to all sequences in the dataset to examine consistency with assignments to sequences that fell within the same 97% similarity cluster.

Representative sequences of each cluster were compared to database sequences in GenBank (NCBI) and UNITE (Kõljalg *et al.* 2013) using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* 1990). Results were compared based on occurrence reports from the Global Biodiversity Information Facility (GBIF.org), known associations with host trees, and morphology. When taxonomic assignments conflicted with sporocarp morphology taxonomic assignments were based on ecological and morphological information as well as on a consensus with BLAST results.

### **Statistical Methods:**

The design of my experiment is a 2x2 factorial randomized complete block design with six blocks (stands) and five repeated measures (collection trips). Multivariate analyses were used to determine community assemblage responses to nutrient additions, an indicator species analysis was used to determine species more abundant under each nutrient condition (+N, +P, +N and P, and control), and linear mixed effect models were used to analyze univariate responses (sporocarp abundance, aboveground EMF biomass, and species richness).

Nonmetric multidimensional scaling (NMDS) was used to visualize community structure within the five collection trips and across the entire season. Data were transformed using the ‘decostand’ function in the R package *vegan* (Dixon 2003; R core team 2018). The data transformation ‘total’ was used to modify the weights of total counts within each sample to profile site-to-site variation. The Bray-Curtis distance measure yielded the greatest fit for the NMDS plots. The ‘Adonis2’ function in *vegan* was used to determine the statistical significance of community responses to N, P, the interaction of N and P, and trip number. Community variation between forests stands was constrained using the command ‘strata’. Canonical analysis of principle coordinates (CAP) was used to visualize community patterns associated with nutrient treatments using the function ‘capscale’ in *vegan*.

An indicator species analysis was used to determine species closely related to the environmental conditions collection date, treatment type, and stand age. Groups were manually constructed based on each of these three environmental conditions, and the function ‘mutipatt’ within the R package *indicspecies* (De Caceres and Legendre 2009) was used to determine the statistical significance of indicator species within each group.).

The R package *lmerTest* (Kuznetsova *et al.* 2017) was used to analyze variance in the data with  $\alpha=0.05$ . Linear mixed effect models were used to determine the effects of N, and P, stand, EM tree basal area, and collection trip on total sporocarp abundance, aboveground fungal biomass, and species richness. Univariate response variables (total abundance, species richness, and biomass) were analyzed with a split-plot in time that kept the plot as the true unit of replication. Plot was treated as a random effect to address the problem of repeated measures caused by multiple collection trips ( $\sim \text{Trip} * \text{P} * \text{N} + \text{stand} + (1|\text{plot})$ ). ANOVA was used to determine the significance

of each effect and denominator degrees of freedom were calculated with the Kenward Roger approximation (Kenward and Roger 1997). All plotting was done using Base R and ggplot2 (Wickham 2016).

## RESULTS

### Fungal Collection and Taxonomic Assignments

In total, 4,570 sporocarps were collected and classified into 35 genera and 103 OTUs (Table 5). Some OTUs were not identifiable either morphologically or molecularly beyond order or family. Those groups are counted based on the highest assigned taxonomic value. The genera *Amanita*, *Cortinarius*, *Lactarius*, and *Russula* were the most species rich and abundant in the collection. One sporocarp from the hypogeous genus *Elaphomyces* was likely unearthed by a foraging animal and was found and added to the collection. Taxonomic names and ranks were determined based on consensus results from naïve Bayes classifier taxonomy, BLAST matched compared between UNITE (Nilsson *et al.* 2018) and GenBank (NCBI), and morphological and ecological traits (Table 6). Previously unidentified morphospecies groups were identified through sequences analyses. Sporocarps that belonged to groups that do not form ectomycorrhizal partnerships with plants were excluded from the analysis but are included for reference in Table 6. Some OTUs did not yield clear taxonomic assignments. Those with clear morphological description are named with comparisons to their closest morphological match (cf.). Groups with uncertain species epithets have been noted with parentheses. Two sequence clusters were assigned the same epithet by both naïve Bayes classifier taxonomy and by best matches when they were subject to BLAST search, but sequences were grouped into two distinct clusters with greater than 3% dissimilarity from each other. They were referred to as *Cortinarius anomalus* var. 1 and *Cortinarius anomalus* var. 2.

**Table 5:** Names of identified fungal genera along with the OTU richness within each genus and the number of sporocarps counted from that genus

<b>Taxonomic Groups (Genus or higher taxonomic assignment)</b>	<b>OTU Richness<sub>1</sub></b>	<b>Sporocarp Abundance</b>	<b>Genus or highest taxonomic assignment (cont.)</b>	<b>OTU Richness<sub>1</sub> (cont.)</b>	<b>Sporocarp Abundance (cont.)</b>
<i>Amanita</i>	13*	718	<i>Laccaria</i>	2	13
<i>Austroboletus</i>	1	13	<i>Lactarius</i>	7*	1220
Boletaceae (family)	1	5	<i>Leccinum</i>	4*	164
Boletales (order)	*	21	<i>Paxillus</i>	1	64
<i>Boletus</i>	2	5	<i>Phylloporus</i>	1	16
<i>Cantharellus</i>	1	12	<i>Pulveroboletus</i>	1	6
<i>Chalciporus</i>	1	4	<i>Ramaria</i>	2	12
<i>Clavulina</i>	1	29	<i>Retiboletus</i>	1	114
<i>Coltricia</i>	2	8	<i>Rhizopogon</i>	1	1
<i>Cortinarius</i>	23*	771	<i>Russula</i>	19*	852
<i>Craterellus</i>	1	25	Russulaceae (family)	*	14
<i>Elaphomyces</i>	1	1	<i>Scleroderma</i>	1	239
<i>Gyroporus</i>	1	13	<i>Strobilomyces</i>	1	39
<i>Hebeloma</i>	1	1	<i>Tylopilus</i>	1*	21
<i>Helvella</i>	2	9	<i>Xanthoconium</i>	1*	67
<i>Hydnum</i>	2	36	<i>Xercomellus</i>	1	22
<i>Hygrophorus</i>	1	13	<i>Xercomus</i>	2	10
<i>Inocybe</i>	3	11	<b>Grand Total</b>	<b>103*</b>	<b>4,570</b>

*Note:* Species groups that were identifiable as distinct OTUs but did not match a described species name are named sp. 1, sp. 2, etc. and are included in species richness counts. Sporocarps that were not identifiable due to poor sample quality are named based on their lowest identifiable taxonomic assignment and are noted as ‘unidentified’. Genera, orders, or families, with an unidentified species category are noted (\*) and this category was excluded from species richness measures.

**Table 6:** Identification of sporocarps in BEF, based on morphology, naïve Bayes classifier taxonomy, and BLAST matches from GenBank

Sample ID <sup>1</sup>	Bases / Seq <sup>2</sup>	Naïve Bayes Classifier Taxonomy <sup>3</sup>	Bases / Cluster <sup>4</sup>	Confidence <sup>5</sup>	BLAST Name & Accession <sup>6</sup>	Bases <sup>7</sup>	Max. Score <sup>8</sup>	Query Cover <sup>9</sup>	E - value <sup>10</sup>	% IDs <sup>11</sup>	Consensus Taxon <sup>12</sup>	GenBank Accession <sup>13</sup>
CNV110	125	Agaricomycetes	786	0.957341787	<i>Ramaria stricta</i> JQ408221.1	1613	1310	100%	0	96.25	<i>Ramaria stricta</i>	MT345282
CNV112	786											
CNV059	227	Agaricales	227	0.759243355	<i>Inocybe tahquamenonensis</i> MK607027.1	670	392	100%	3.00E-105	97.37%	<i>Inocybe</i> cf. <i>tahquamenonensis</i>	MT345242
CNV034	807	<i>Amanita</i> unidentified	807	1	<i>Amanita rubescens</i> AJ889923.1	826	1391	100%	0	97.77%	<i>Amanita</i> cf. <i>rubescens</i>	MT345253
CNV042	739	<i>Amanita bisporigera</i>	739	0.992365956	<i>Amanita bisporigera</i> KJ638292.1	619	1122	82%	0	99.84%	<i>Amanita bisporigera</i>	MT345272
CNV033	197	<i>Amanita brunnescens</i>	826	1	<i>Amanita brunnescens</i> KT006762.1	776	1432	93%	0	100%	<i>Amanita brunnescens</i>	MT345189
CNV038	826											
CNV040	821											
CNV044	820											
CNV039	298	<i>Amanita lavendula</i>	301	0.99947405	<i>Amanita lavendula</i> JF313664.1	644	239	99%	2.00E-59	100%	<i>Amanita citrina</i> var. <i>lavendula</i>	MT345218
CNV178	301											
CNV036	782	<i>Amanita flavoconia</i>	797	0.999967119	<i>Amanita flavoconia</i> MK580711.1	693	1247	84%	0	100%	<i>Amanita flavoconia</i>	MT345206
CNV037	797											
CNV021	704	<i>Amanita fulva</i>	397	0.999986956	<i>Amanita fulva</i> MN755843.1	2020	734	100%	0	100%	<i>Amanita fulva</i>	MT345213
CNV024	397											
CNV029	258	<i>Amanita jacksonii</i>	258	0.999983107	<i>Amanita jacksonii</i> MH281889.1	586	451	100%	6.00E-123	98.08%	<i>Amanita jacksonii</i>	MT345243
CNV026	594	<i>Amanita muscaria</i>	594	0.999110093	<i>Amanita muscaria</i> GQ250402.1	746	1062	99%	0	98.99%	<i>Amanita muscaria</i>	MT345275
CNV023	680	<i>Amanita olivaceogrisea</i>	696	0.916808291	<i>Amanita olivaceogrisea</i> MT073014.1	1012	1280	100%	0	99.86%	<i>Amanita olivaceogrisea</i>	MT345201
CNV031	712											
CNV043	696											

CNV030	809	<i>Amanita porphyria</i>	809	0.999993497	<i>Amanita porphyria</i> HM196012.1	1048	1489	100%	0	99.88%	<i>Amanita porphyria</i>	MT345267
CNV022	767	<i>Amanita virosa</i>	767	0.999989207	<i>Amanita virosa</i> MG516218.1	2018	1332	98%	0	98.30%	<i>Amanita virosa</i>	MT345282
CNV041	778	<i>Amanita volvata</i>	778	0.999999764	<i>Amanita volvata</i> JF723273.1	768	1264	96%	0	96.95%	<i>Amanita volvata</i>	MT345230
C6.227	Failed	Failed		Failed							<i>Amanita cf. constricta</i> <sup>14</sup>	
CNV113	592	<i>Clavulinopsis umbrinella</i>	592	0.701762774	<i>Clavulinopsis</i> sp. MK607506.1	650	689	100%	0	88.00%	<i>Clavulinopsis</i> unidentified <sup>15</sup>	MT345232
CNV114	600	<i>Clavulinopsis</i> unidentified	581	1	<i>Clavulinopsis</i> sp. MH399871.1	582	1016	98%	0	98.61%	<i>Clavulinopsis</i> unidentified <sup>15</sup>	MT345223
CNV124	581											
CNV115	568	Clavariaceae	568	0.987041546	<i>Ramariopsis crocea</i> MK607557.1	637	507	100%	3.00E-139	83.48%	<i>Ramariopsis</i> <sup>15</sup>	MT345227
CNV183	506	<i>Cortinarius</i> unidentified	773	0.999978533	<i>Cortinarius rigens</i> GQ159900.1	1217	1301	99%	0	97.03%	<i>Cortinarius</i> sp. 1	MT345185
CNV199	508											
CNV204	769											
CNV233	465											
CNV234	608											
CNV218	678	<i>Cortinarius</i> unidentified	678	0.998797501	<i>Cortinarius fasciatus</i> GQ159913.1	1134	1103	100%	0	96.17%	<i>Cortinarius</i> sp. 2	MT345240
CNV201	776	<i>Cortinarius</i> unidentified	786	0.999417601	<i>Cortinarius</i> sp. MG982536.1	816	1426	98%	0	99.74%	<i>Cortinarius azureus</i>	MT345216
CNV202	786											
CNV102	462	<i>Cortinarius</i> unidentified	462	0.999978057	<i>Cortinarius laetissimus</i> GQ159898.1	1141	811	100%	0	98.28%	<i>Cortinarius</i> sp. 3	MT345265
CNV184	690	<i>Cortinarius alboviolaceus</i>	442	0.994953238	<i>Cortinarius alboviolaceus</i> MH784679.1	618	780	100%	0	100%	<i>Cortinarius alboviolaceus</i>	MT345181
CNV209	696											
CNV214	422											
CNV215	695											
CNV236	658											

CNV241	691											
CNV196	690	<i>Cortinarius annulatus</i>	690	1	<i>Cortinarius tofaceus</i> KU236707.1	791	1273	99%	0	100%	<i>Cortinarius annulatus</i>	MT345249
CNV192	760	<i>Cortinarius anomalovelatus</i>	760	0.999373707	<i>Cortinarius anomalovelatus</i> FJ717605.1	1264	1321	100%	0	98.16%	<i>Cortinarius anomalovelatus</i>	MT345235
CNV219	298	<i>Cortinarius anomalus</i>	769	0.999088668	<i>Cortinarius rigens</i> GQ159900.1	1217	1284	100%	0	96.76%	<i>Cortinarius anomalus</i> var. 1	MT345184
CNV229	475											
CNV246	773											
CNV248	769											
CNV249	729											
CNV230	478	<i>Cortinarius anomalus</i>	612	0.999088668	<i>Cortinarius anomalus</i> KY595995.1	772	1120	100%	0	99.67%	<i>Cortinarius anomalus</i> var. 2	MT345186
CNV231	456											
CNV232	612											
CNV242	777											
CNV245	637											
CNV203	699	<i>Cortinarius bivelus</i>	699	0.910552199	<i>Cortinarius bivelus</i> AY669682.1	1136	1230	98%	0	98.98%	<i>Cortinarius bivelus</i>	MT345199
CNV217	696											
CNV244	623											
CNV188	715	<i>Cortinarius emunctus</i>	715	0.999999943	<i>Cortinarius salor</i> FJ039600.1	1189	1273	100%	0	98.88%	<i>Cortinarius salor</i>	MT345255
CNV211	433	<i>Cortinarius erubescens</i>	433	0.860752386	<i>Cortinarius roseobasilis</i> KU041741.1	629	763	100%	0	98.39%	<i>Cortinarius (erubescens)</i>	MT345257
CNV190	735	<i>Cortinarius illibatus</i>	735	0.999999481	<i>Cortinarius delibutus</i> AJ236065.2	676	1151	87%	0	98.92%	<i>Cortinarius delibutus</i>	MT345178
CNV191	605											
CNV207	790											
CNV208	465											
CNV213	797											
CNV227	467											
CNV205	796	<i>Cortinarius laniger</i>	818	0.999999867	<i>Cortinarius laniger</i> GQ159857.1	1231	1452	97%	0	99.50%	<i>Cortinarius laniger</i>	MT345224
CNV206	818											



CNV228	512	<i>Cortinarius</i>	512	0.99991566	<i>Cortinarius</i>	552	946	100%	0	100%	<i>Cortinarius</i>	MT345210
CNV250	666	<i>leiocastaneus</i>			<i>leiocastaneus</i>						<i>leiocastaneus</i>	
					NR_119678							
CNV189	703	<i>Cortinarius</i>	703	0.999995149	<i>Cortinarius</i>	1230	1293	100%	0	99.86%	<i>Cortinarius</i>	MT345269
		<i>pholideus</i>			<i>pholideus</i>						<i>pholideus</i>	
					AY669694.1							
CNV223	465	<i>Cortinarius</i>	465	0.808742459	<i>Cortinarius</i>	653	859	100%	0	100%	<i>Cortinarius</i>	MT345254
		<i>porphyropus</i>			<i>porphyropus</i>						<i>porphyropus</i>	
					AJ236069.2							
CNV226	745	<i>Cortinarius talus</i>	745	0.994869394	<i>Cortinarius talus</i>	1317	1358	100%	0	99.60%	<i>Cortinarius talus</i>	MT345236
					KJ421141.1							
CNV210	696	<i>Cortinarius</i>	697	0.997235856	<i>Cortinarius torvus</i>	730	1181	100%	0	97.29%	<i>Cortinarius torvus</i>	MT345208
CNV120	697	<i>torvus</i>			AJ889977.1							
CNV186	346	<i>Cortinarius</i>	669	0.999996387	<i>Cortinarius valgus</i>	631	1024	82%	0	100%	<i>Cortinarius</i>	MT345182
		<i>valgus</i>			KF961225.1						<i>(valgus)</i>	
CNV187	638											
CNV243	692											
CNV252	669											
CNV237	315	<i>Cortinarius</i>	315	0.999251262	<i>Cortinarius</i>	1546	582	100%	3.00E-162	100%	<i>Cortinarius</i>	MT345278
		<i>violaceus</i>			<i>violaceus</i>						<i>violaceus</i>	
					KY964825.1							
CNV198	784	<i>Cortinarius</i>	784	0.76575623	<i>Cortinarius</i> sp.	1260	1339	100%	0	97.58%	<i>Cortinarius</i>	MT345274
		<i>xanthocephalus</i>			FJ039656.1						<i>(xanthocephalus)</i> <sup>16</sup>	
CNV195	Failed	Failed									<i>Cortinarius</i>	
CNV224	Failed										<i>armillatus</i> <sup>14</sup>	
CNV239	Failed	Failed									<i>Cortinarius</i>	
											<i>flexipes</i> <sup>14</sup>	
CNV200	Failed	Failed									<i>Cortinarius</i>	
											<i>iodes</i> <sup>14</sup>	
CNV136	671	<i>Coltricia</i>	671	1	<i>Coltricia perennis</i>	763	1195	96%	0	100%	<i>Coltricia perennis</i>	MT345222
CNV137	914	<i>perennis</i>			KU360688.1							
CNV138	724	<i>Coltricia weii</i>	724	0.957337578	<i>Coltricia</i>	704	1186	96%	0	97.42%	<i>Coltricia</i> cf.	MT345226
					<i>subperennis</i>						<i>cinnamomea</i> <sup>14</sup>	
					KY693736.1							
CNV173	750	<i>Craterella fallax</i>	750	0.999992945	<i>Craterella fallax</i>	901	1386	100%	0	100%	<i>Craterella fallax</i>	MT345207
CNV174	661				GU590927.1							

CNV130	485	485		<i>Cudonia</i> sp. KC833133.1	510	828	93%	0	99.56%	<i>Cudonia</i> unidentified <sup>15</sup>	Not submitted	
CNV163	790	790		<i>Gliophorus</i> <i>irrigatus</i> KF291086.1	643	1177	81%	0	99.69%	<i>Gliophorus</i> <i>irrigatus</i> <sup>15</sup>	Not submitted	
C1.099	Failed	Failed								<i>Cantharellus</i> <i>flavus</i> <sup>14</sup>		
CNV164	783	<i>Entoloma</i> <i>luridum</i>	783	1	<i>Entoloma luridum</i> KC710080.1	714	1218	85%	0	99.40%	<i>Entoloma</i> <i>luridum</i> <sup>15</sup>	MT345263
CNV121	522	<i>Entoloma</i> unidentified	522	0.876398282	<i>Entoloma</i> <i>porphyrophaeum</i> MN906139.1	683	767	80%	0	99.53%	<i>Entoloma</i> sp. 2 <sup>15</sup>	MT345239
CNV119	489	<i>Elaphomyces</i> <i>granulatus</i>	489	0.999742763	<i>Elaphomyces</i> <i>granulatus</i> KX238852.1	661	885	100%	0	99.39%	<i>Elaphomyces</i> <i>granulatus</i>	MT345264
CNV253	777	<i>Hebeloma</i> unidentified	777	0.981557264	<i>Hebeloma</i> sp. DQ822807.1	819	1417	100%	0	99.49%	<i>Hebeloma</i> sp. 1	MT345229
CNV162	661	<i>Inocybe</i> unidentified	661	0.988821454	<i>Inocybe</i> cf. <i>rimosa</i> JQ408775.1	717	1044	86%	0	99.65%	<i>Inocybe</i> cf. <i>rimosa</i>	MT345228
CNV108	753	<i>Inocybe</i> <i>tubarioides</i>	753	1	<i>Inocybe</i> <i>tubarioides</i> MH594211.1	681	1232	88%	0	100%	<i>Inocybe</i> <i>tubarioides</i>	MT345276
CNV133	689	<i>Helvella</i> <i>lacunosa</i>	689	0.860415098	<i>Helvella lacunosa</i> KT894823.1	745	920	96%	0	91.69%	<i>Helvella lacunosa</i>	MT345260
CNV117	568	<i>Helvella</i> <i>macropus</i>	932	1	<i>Helvella macropus</i> MG773828.1	922	1664	97%	0	99.78%	<i>Helvella macropus</i>	MT345251
CNV158	472	<i>Hydnum</i> unidentified	473	0.999999489	<i>Hydnum</i> <i>cuspidatum</i> MK282424.1	663	874	100%	0	100%	<i>Hydnum</i> <i>cuspidatum</i>	MT345209
CNV159	273											
CNV157	736	<i>Hydnum</i> unidentified	736	0.781626906	<i>Hydnum repandum</i> AY817136.1	812	1264	100%	0	97.69%	<i>Hydnum</i> <i>repandum</i>	MT345247
CNV122	780	<i>Laccaria</i> unidentified	780	0.998806783	<i>Laccaria bicolor</i> FJ845417.1	971	1424	100%	0	99.62%	<i>Laccaria bicolor</i>	MT345252
CNV123	783	<i>Laccaria</i> unidentified	783	0.999910488	<i>Laccaria</i> sp. JX030275.1	776	1424	98%	0	100%	<i>Laccaria</i> cf. <i>striatula</i>	MT345281
CNV240	777	<i>Pholiota lenta</i>	777	0.999983644	<i>Pholiota lenta</i> MT075528.1	1131	1421	100%	0	99.61%	<i>Pholiota lenta</i> <sup>15</sup>	MT345248

CNV134	608	<i>Paxillus involutus</i>	857	1	<i>Paxillus involutus</i> EU486436.1	1350	1572	100%	0	99.65%	<i>Paxillus involutus</i>	MT345211
CNV135	857											
CNV046	397	<i>Scleroderma citrinum</i>	397	0.999992912	<i>Scleroderma citrinum</i> MH930125.1	714	734	100%	0	100%	<i>Scleroderma citrinum</i>	MT345233
CNV126	173		607	0.999903688		699	1116	100%	0	99.84%		
CNV127	607	<i>Clavulina cinerea</i>			<i>Clavulina cinerea</i> MH979319.1						<i>Clavulina cinerea</i>	MT345192
CNV128	594											
CNV129	785											
CNV111	790	Gomphaceae	790	1	<i>Ramaria</i> sp. DQ365605.1	731	1245	85%	0	99.85%	<i>Ramaria</i> sp. 1	MT345245
CNV002	795	<i>Lactarius camphoratus</i>	844	0.99711798	<i>Lactarius rimosellus</i> KU518879.1	743	1306	86%	0	99.17%	<i>Lactarius camphoratus</i>	MT345221
CNV016	844											
CNV010	821	<i>Lactarius cinereus</i>	856	0.999904137	<i>Lactarius cinereus</i> FJ348708.1	1369	1557	100%	0	99.53%	<i>Lactarius cinereus</i>	MT345202
CNV015	835											
CNV018	856											
CNV260	620	<i>Lactarius fuliginosus</i>	620	0.999827421	<i>Lactarius fumosibrunneus</i> JN797632.1	1265	1138	100%	0	99.68%	<i>Lactarius fuliginosus</i>	MT345277
CNV013	823	<i>Lactarius hysginus</i>	823	0.996026952	<i>Lactarius</i> sp. MH985025.1	689	1251	83%	0	99.42%	<i>Lactarius hysginus</i>	MT345191
CNV019	344											
CNV001	843	<i>Lactarius tabidus</i>	843	0.957647923	<i>Lactarius tabidus</i> KP783447.1	792	1365	94%	0	97.74%	<i>Lactarius tabidus</i>	MT345259
CNV006	808	<i>Lactarius torminosus</i>	808	0.985867781	<i>Lactarius torminosus</i> DQ367908.1	1018	1483	100%	0	99.75%	<i>Lactarius torminosus</i>	MT345220
CNV007	819											
CNV003	446	<i>Lactarius vinaceorufescens</i>	697	0.999830435	<i>Lactarius vinaceorufescens</i> KF241542.1	717	1279	99%	0	99.86%	<i>Lactarius vinaceorufescens</i>	MT345196
CNV017	449											
CNV105	697											
CNV011	Failed	Failed									<i>Lactarius lignyotus</i> <sup>14</sup>	
CNV275	765	<i>Russula</i> unidentified	765	1	<i>Russula laccata</i> HQ604844.1	1352	1393	100%	0	99.48%	<i>Russula</i> sp. 1	MT345246

CNV297	329	<i>Russula</i> unidentified	329	0.939535603	<i>Russula rutila</i> KY582724.1	619	538	97%	6.00E-149	96.90%	<i>Russula</i> sp. 2	MT345262
CNV300	709	<i>Russula</i> unidentified	709	0.997081115	<i>Russula</i> sp. MH212105.1	590	767	66%	0	98.18%	<i>Russula</i> sp. 3	MT345273
CNV272	380	<i>Russula</i> unidentified	380	0.752833034	Russulaceae sp. AB831843.1	774	658	100%	0	97.89%	<i>Russula</i> sp. 4	MT345241
CNV259	597	<i>Russula</i> unidentified	614	0.956549172	<i>Russula</i> sp. GU220376.1	672	1110	100%	0	99.35%	<i>Russula</i> sp. 5	MT345194
CNV268	781											
CNV271	763											
CNV301	614											
CNV274	794	<i>Russula</i> unidentified	794	0.97190757	<i>Russula</i> sp. AF349711.1	707	1188	84%	0	98.81%	<i>Russula</i> sp. 6	MT345266
CNV267	622	<i>Russula brunneoviolacea</i>	622	1	<i>Russula brunneoviolecea</i> MG687327.1	792	1149	100%	0	100%	<i>Russula brunneoviolacea</i>	MT345258
CNV270	781	<i>Russula crustosa</i>	764	0.999115422	<i>Russula crustosa</i> KM373243.1	754	1339	96%	0	99.46%	<i>Russula crustosa</i>	MT345187
CNV279	794											
CNV280	802											
CNV281	764											
CNV286	597											
CNV160	527	<i>Russula densifolia</i>	527	0.999998536	<i>Russula densifolia</i> MG687332.1	779	917	100%	0	98.10%	<i>Russula densifolia</i>	MT345271
CNV167	777	<i>Russula dissimulans</i>	777	0.999999294	<i>Russula nigricans</i> KC581314.1	1206	1330	99%	0	97.68%	<i>Russula dissimulans</i>	MT345234
CNV298	742	<i>Russula fellea</i>	742	0.999998611	<i>Russula fellea</i> KF245536.1	746	1267	95%	0	98.74%	<i>Russula fellea</i> <sup>16</sup>	MT345237
CNV262	766	<i>Russula granulata</i>	766	0.999999731	<i>Russula granulata</i> JQ272365.1	712	1206	85%	0	100%	<i>Russula granulata</i>	MT345238
CNV263	727	<i>Russula grata</i>	781	0.992894861	<i>Russula</i> cf. <i>lauroceraci</i> KF245507.1	785	1415	99%	0	99.49%	<i>Russula grata</i>	MT345250
CNV278	781											
CNV273	666	<i>Russula heterophylla</i>	666	0.999989887	<i>Russula grisea</i> KX963792.1	1207	1173	99%	0	98.64%	<i>Russula (vesca)</i>	MT345183
CNV276	614											
CNV277	642											

CNV285	755												
CNV294	754												
CNV284	771	<i>Russula rosea</i>	791	0.953612859	<i>Russula lepida</i> MG687359.1	737	1243	93%	0	97.02%	<i>Russula</i> cf. <i>claroflava</i> <sup>17</sup>	MT345180	
CNV287	779												
CNV288	738												
CNV289	791												
CNV302	721												
CNV303	724												
CNV261	796	<i>Russula sphagnophila</i>	764	0.999911305	<i>Russula nitida</i> MG687360.1	787	1384	98%	0	99.74%	<i>Russula sphagnophila</i>		MT345217
CNV295	764												
CNV256	742	<i>Russula subsulphurea</i>	809	0.999484431	<i>Russula</i> sp. JQ272402.1	748	1284	85%	0	100%	<i>Russula rugulosa</i> <sup>17</sup>	MT345197	
CNV257	614												
CNV258	788												
CNV290	809												
CNV291	809												
CNV296	757												
CNV282	Failed	Failed											<i>Russula brevipes</i> <sup>14</sup>
CNV283	Failed												
C4.006	Failed	Failed										<i>Russula paludosa</i> <sup>14</sup>	
C9.001	Failed	Failed										<i>Russula silvicola</i> <sup>14</sup>	
C8.224	Failed	Failed										Boletales unidentified <sup>14</sup>	
CNV139	Failed	Failed										<i>Hygrophorus (picea)</i> <sup>14</sup>	
CNV089	695	Boletales	695	0.996418556	Boletales sp. KY826023.1	562	1029	80%	0	99.82%	Boletales unidentified	MT345225	
CNV180	365	Boletales	365	0.999710967	Boletales sp. KY825964.1	422	619	93%	2.00E-173	99.13%	<i>Retiboletus ornatipes</i> <sup>14</sup>	MT345268	
CNV144	862	Boletaceae	855	0.999890487	<i>Xercomellus chrysenteron</i> DQ533981.1	1220	1517	100%	0	98.60%	<i>Xercomellus</i> sp. 1	MT345200	
CNV146	855												
CNV154	863												

CNV051	773	Boletaceae	773	0.989287346	<i>Pulveroboletus rubroscabrosus</i> KX453816.1	620	1014	79%	0	96.44%	<i>Pulveroboletus ravenelii</i> <sup>1</sup>	MT345219
CNV181	747											
CNV076	666	<i>Boletus edulis</i>	666	0.953474375	<i>Boletus</i> sp. KY826155.1	742	1218	100%	0	99.55%	<i>Boletus edulis</i>	MT345279
CNV087	823	<i>Boletus subvelutipes</i>	858	0.999999949	<i>Boletus subvelutipes</i> MH244205.1	750	1386	87%	0	100%	<i>Boletus subvelutipes</i>	MT345190
CNV088	858											
CNV093	818											
CNV165	837											
CNV077	744	<i>Imleria badia</i>	726	0.999837139	<i>Xercomus badius</i> HQ207696.1	761	1242	100%	0	97.80%	<i>Imleria badia</i>	MT345215
CNV092	726											
CNV063	879	<i>Leccinum</i> unidentified	878	1	<i>Leccinum scabrum</i> JF899566.1	600	1098	90%	0	99.67%	<i>Leccinum</i> sp. 1	MT345195
CNV064	195											
CNV065	307											
CNV071	878											
CNV142	953											
CNV069	840	<i>Leccinum scabrum</i>	530	0.946882632	<i>Leccinum holopus</i> AF454562.1	1486	963	100%	0	99.43%	<i>Leccinum holopus</i>	MT345188
CNV072	530											
CNV099	417											
CNV140	664											
CNV062	621	<i>Leccinum scabrum</i>	621	0.939604779	<i>Boletus</i> sp. KY826141.1	922	1120	100%	0	99.20%	<i>Leccinum scabrum</i>	MT345214
CNV068	925											
CNV066	911	<i>Leccinum versipelle</i>	911	0.984953735	<i>Leccinum versipelle</i> AF454574.1	1430	1655	98%	0	99.89%	<i>Leccinum versipelle</i>	MT345270
CNV179	965	<i>Phylloporus leucomycelinus</i>	965	1	<i>Phylloporus leucomycelinus</i> JQ967249.1	832	1528	86%	0	99.76%	<i>Phylloporus leucomycelinus</i>	MT345256
CNV049	587	<i>Strobilomyces strobilaceus</i>	587	1	<i>Strobilomyces</i> aff. <i>strobilaceus</i> JQ318964.1	576	833	76%	0	100%	<i>Strobilomyces strobilaceus</i>	MT345280
CNV056	432	<i>Tylopilus felleus</i>	410	1	<i>Tylopilus rubrobrunneus</i> MK560151.1	450	752	99%	0	100%	<i>Tylopilus felleus</i>	MT345204
CNV058	410											
CNV091	788											

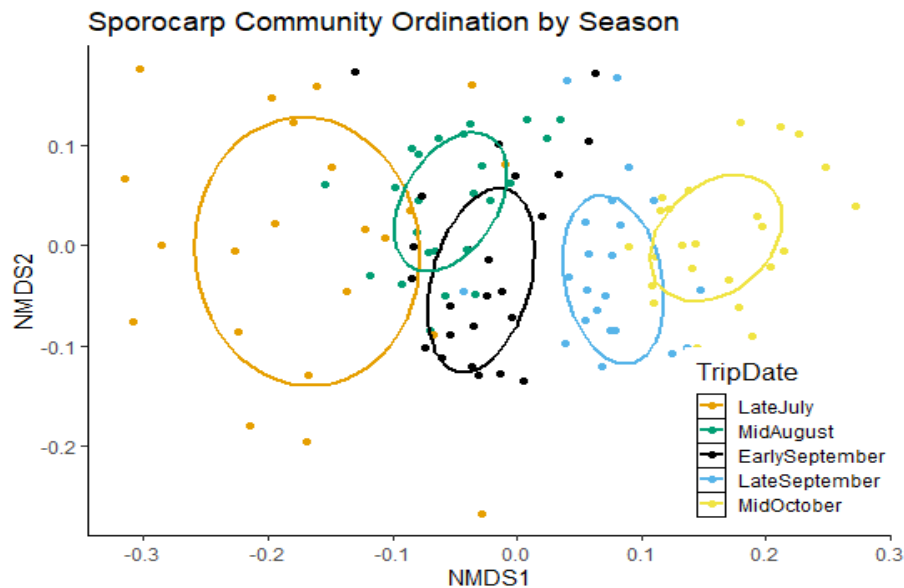
CNV054	383	<i>Tylopilus</i> unidentified	383	0.854387127	Boletales sp. KY826028.1	789	708	100%	0	100%	<i>Tylopilus</i> sp. 1	MT345261
CNV075	488	Boletaceae unidentified	488	0.912572529	Boletales sp. KY826075.1	632	745	87%	0	97.42%	Boletaceae unidentified 3	MT345198
CNV182	470											
CNV048	847	Boletaceae unidentified	847	0.999608652	<i>Austroboletus</i> <i>gracilis</i> MH979242.1	733	1315	84%	0	99.58%	<i>Austroboletus</i> <i>gracilis</i>	MT345212
CNV151	826											
CNV097	365	Boletaceae unidentified	588	0.948529681	<i>Leccinum albellum</i> MH488723.1	697	1077	99%	0	99.83%	<i>Leccinum albellum</i>	MT345193
CNV141	482											
CNV143	588											
CNV057	790	Boletaceae unidentified	751	0.999071517	<i>Tylopilus felleus</i> HM190015.1	711	1245	90%	0	99.85%	<i>Tylopilus</i> sp. 2 <sup>18</sup>	MT345203
CNV078	751											
CNV090	720											
CNV079	751	<i>Xanthoconium</i> unidentified	733	0.996490699	<i>Xanthoconium</i> cf. <i>affine</i> FJ480435.1	719	1295	96%	0	99.72%	<i>Xanthoconium</i> sp. 1	MT345179
CNV085	384											
CNV096	721											
CNV145	733											
CNV149	731											
CNV150	734											
CNV074	799	<i>Xercomus</i> unidentified	799	0.957326822	<i>Xercomus</i> <i>ferrugineus</i> HQ207698.1	771	1336	96%	0	97.93%	<i>Xercomus</i> sp. 1	MT345231
CNV084	839	<i>Chalciporus</i> <i>piperatus</i>	843	1	<i>Chalciporus</i> <i>piperatus</i> AF335457.1	854	1434	100%	0	97.29%	<i>Chalciporus</i> <i>piperatus</i>	MT345205
CNV148	843											
CNV053	739	<i>Gyroporus</i> <i>cyanescens</i>	739	0.989605057	Boletales sp. KY826067.1	516	898	69%	0	97.87%	<i>Gyroporus</i> <i>cyanescens</i>	MT345244

- 
1. Sample ID: unique number assigned to voucher specimen. OTUs with multiple sample ID's reflect redundant sequences.
  2. Bases / Sequence: length in base pairs of individual sequences prior to clustering at 97%
  3. Naïve Base Classifier Taxonomy: Taxonomy assigned to 97% cluster using the QIIME formatted UNITE dynamic species hypothesis dataset (version 8.0, released 02.02.2019; Kõljalg *et al.* 2013)
  4. Bases / Cluster: length in base pairs of representative sequences for each cluster
  5. Confidence: confidence value associated with naïve base classifier taxonomic assignment.
  6. BLAST name and accession: best match to query search in GenBank and associated accession number
  7. Bases: length of sequence of best BLAST match
  8. Max. score: assigned score based on quality. Scores higher than 1000 are ideal.
  9. Query coverage (%): comparison of query sequence length to closest BLAST match.
  10. E-Value: the probability of seeing the sequence matching as a result of random chance.
  11. Percent identity: percent of bases that match between subject and query sequence.
  12. Consensus taxon: finalized identification of sample based on morphological and molecular evidence
  13. GenBank Accession: accession number under which the ITS region of the sample was submitted to GenBank.
  14. Consensus ID assigned based on morphological description due to poor sequence turn out.
  15. Non mycorrhizal or ambiguous ecology, included in table for reference but left out of analysis.
  16. Consensus name based on best match morphologically and genetically but low occurrence of epithet species in North America.
  17. Taxonomic assignments based on naïve base classifier and best database matches do not match morphological description. Consensus ID based on morphological description
  18. Morphologically similar to *Tylopilus felleus* samples (CNV056, CNV058, and CNV091), but sequences differed >3%
-



## Sporocarp Community Composition

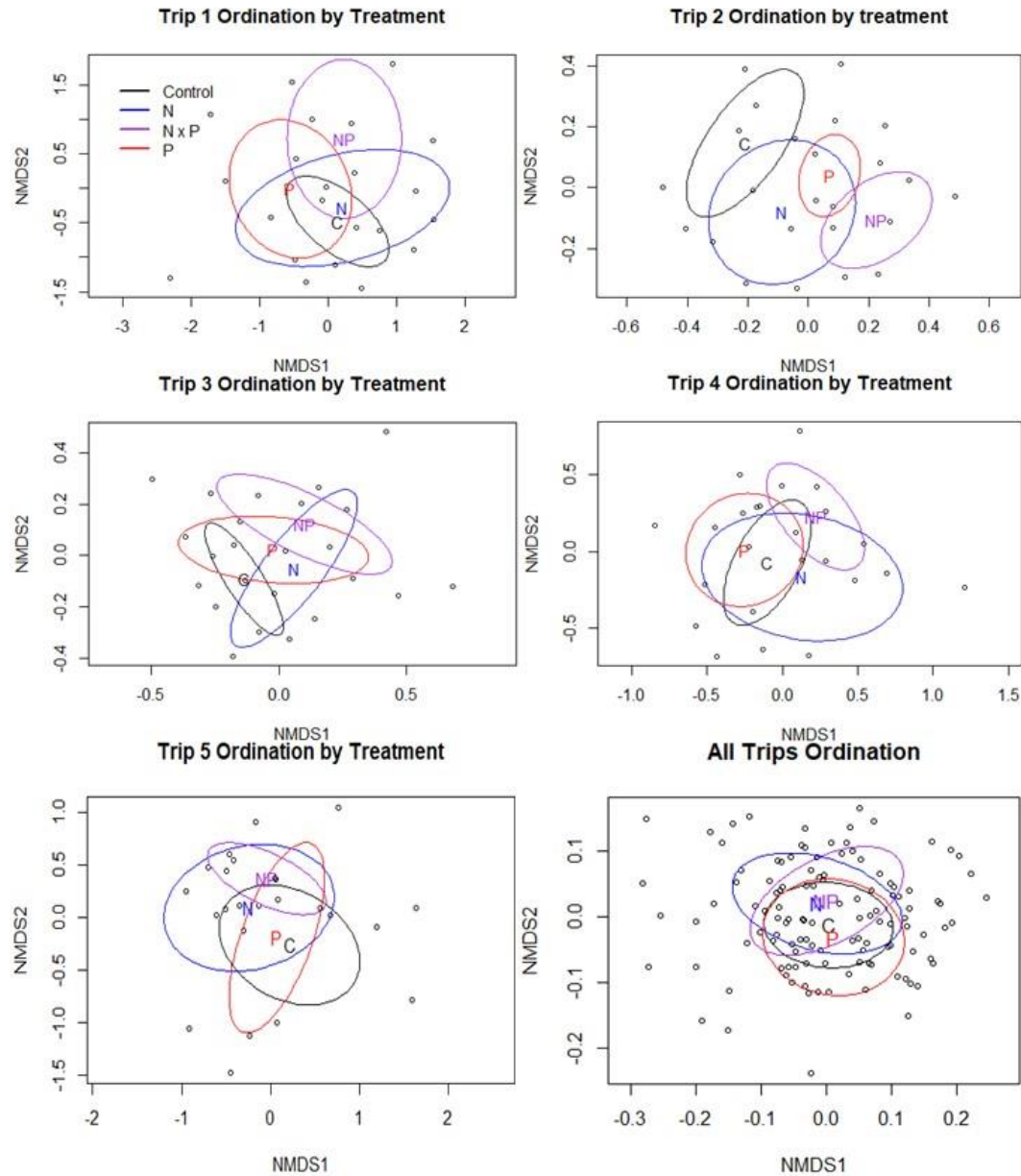
Different fungal species produced sporocarps at different times throughout the fruiting season (Figure 3). Seasonality was the primary explanatory variable for variation in sporocarp community composition, and fungal fruiting patterns shifted linearly across the season ( $p < 0.001$ ). Ectomycorrhizal sporocarp composition responded to N and P additions. Plots treated with nitrogen had different fungal communities than those in control plots ( $p = 0.001$ ). Plots treated with P also varied in community composition compared to control plots ( $p = 0.001$ ).



**Figure 3:** Sporocarp community composition across each of the five collection trips ranging from late July-mid October. Ellipses represent individual collections and their size reflects the standard deviation from the center point. Ellipses that do not overlap are generally considered distinct communities and these groups were confirmed using Adonis2. The primary axis (NMDS1) represented most of the variation within the plot.

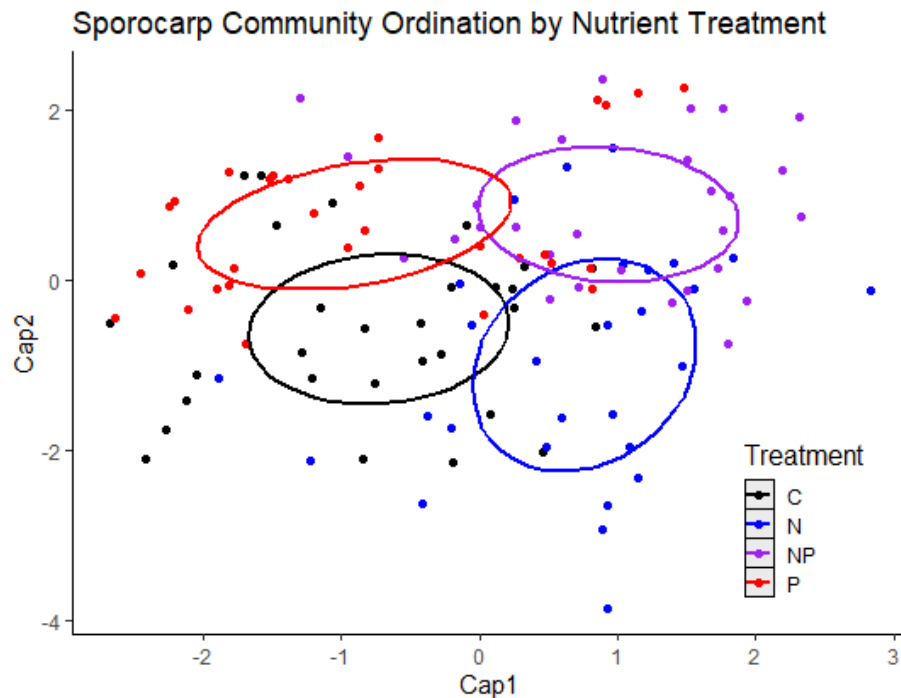
Sporocarp community composition changes between treatments were not consistent within each trip. Trips two and three, which occurred in mid-August and early September respectively, yielded the greatest sporocarp abundance. Community responses to nutrients were the more distinct during these collection trips relative to those earlier or later in the season (Figure 4). Within

these trips sporocarp community composition was more dissimilar from control in plots treated with both nitrogen and phosphorus (N+P) than in either N or P plots. Each trip was modeled with NMDS (Figure 4) and combined trips were modeled with NMDS and CAP (Figures 4 and 5).



**Figure 4:** NMDS plots representing sporocarp community composition within each of the five collection trips. Trip 1 refers to the collection in late July, trip 2 refers to the collection in mid-August, trip 3 refers to the collection in early September, trip 4 refers to the collection in late September and trip 5 refers to the collection in mid-October.

When data were plotted again using CAP with variation due to seasonal changes constrained, community responses to nutrient were clear. The primary (horizontal) axis, which demonstrates the greatest variation in the matrix, was explained by the addition of N ( $p < 0.04$ ; Figure 5). The secondary (vertical) axis reflected the effect of P ( $p = 0.02$ ). While N and P plots are each dissimilar from control plots, N+P plots are more dissimilar from control plots than when either nutrient is added alone suggesting an additive effect on sporocarp community composition. These patterns showed different assemblage structures of EM fungal fruiting in each nutrient condition.



**Figure 5:** Variation between nutrient treatment plots was visualized in a constrained ordination. Each color represents a different nutrient condition where black ellipses and points represent control plots, blue represents N addition plots, purple represents N+P addition plots and red represents P addition plots. Axes represent significant variation in community structure (CAP1  $p > 0.03$ , CAP2  $p = 0.02$ ). Community assemblage shifted right along the X axis in response to N addition and up along the Y axis in response to P addition. Each ellipsis reflects a sporocarp assemblage that was distinct from the other groupings and these grouping were statistically significant.

## Indicator Species

An indicator species analysis determined species whose presence were statistically significantly more abundant at different levels across three different environmental conditions, collection date, stand age, and nutrient condition.

While *Scleroderma citrinum* ( $p < 0.02$ ) and *Russula rugulosa* ( $p < 0.05$ ) fruited constantly throughout the season, other species fruited more abundantly in the early or late season. Early collection trips had more sporocarps from Boletales, and the genus *Amanita*. Specifically, *Austroboletus gracilis* ( $p < 0.01$ ), *Amanita porphyria* ( $p < 0.04$ ), and *Amanita flavoconia* ( $p < 0.01$ ) fruited more abundantly during the first two collection trips (late July and mid-August). *Cortinarius* sp. 1, which can be compared most closely (both morphologically and genetically) to *Cortinarius rigens* was the only species significantly more abundant in the late season ( $p < 0.02$ ). Many species within the genera *Cortinarius* and *Russula* fruited more abundantly in mid-aged stands whereas species within the order Boletales fruited significantly more abundantly in mature stands (Table 8).

Species that fruited more abundantly in different nutrient treatment plots were considered indicator species for different nutrient additions (Table 7). Some taxa that were dominant in the data, including *Lactarius*, *Leccinum*, and *Tylopilus* were not indicator species, suggesting that those groups did not shift significantly in fruiting abundance in response to N or P additions. *Xanthoconium* sp. 1 ( $p < 0.04$ ) and *Austroboletus gracilis* ( $p < 0.04$ ) fruited significantly more abundantly in control plots relative to those with added nutrients. Sporocarps of two species within the genus *Cortinarius* were more abundant in plots with added P than in plots without, and while these results were consistent in each iteration of the model, they were not statistically significant

at  $\alpha=0.05$ . ‘Unidentified *Russula*’ was the only group that fruited significantly more abundantly in P treated plots ( $p<0.04$ ), but this group does not reflect one defined OTU. None of the sporocarps collected were significantly more abundant plots with added nitrogen.

**Table 7:** Indicator species of the nutrient addition plots. Indicator species are those that fruited more abundantly in a specified nutrient condition. Four groups were used to test for indicator species of different nutrient conditions. These groups represent control plot and plots with added N, N+P, or P. Only significant results are shown. Asterisks indicate significance at  $\alpha=0.05$ .

Nutrient Condition	Species	P value
Control	<i>Xanthoconium</i> sp.1	$p<0.04^*$
	<i>Austroboletus gracilis</i>	$p<0.04^*$
	<i>Amanita brunnescens</i>	$p<0.09$
+ N	<i>Amanita virosa</i>	$p<0.08$
+ P	<i>Cortinarius valgus</i>	$p<.10$
	<i>Cortinarius pholideus</i>	$p<0.08$
	<i>Russula</i> unidentified	$p<0.04^*$
	<i>Amanita fulva</i>	$p<0.09$
+ N & P	<i>Paxillus involutus</i>	$p<0.09$

**Table 8:** Indicator species associates with mid-aged and mature forest stands. Mid-aged and mature stands were dominated by different fungal taxa. Asterisks indicate significance at  $\alpha=0.05$ .

Mid aged stands	P value	Mature stands	P value
<i>Cortinarius anomalus</i> var. 1	$p=0.001^*$	<i>Lactarius cinereus</i>	$p=0.0004^*$
<i>Cortinarius valgus</i>	$p<0.001^*$	<i>Scleroderma citrinum</i>	$p<0.001^*$
<i>Russula</i> sp. 5	$p<0.007^*$	<i>Russula</i> sp. 4	$p<0.04^*$
<i>Cortinarius delibutus</i>	$p<0.001^*$	<i>Tylopilus felleus</i>	$p<0.04^*$
<i>Cortinarius alboviolaceus</i>	$p<0.01^*$	<i>Russula</i> cf. <i>claroflava</i>	$p<0.07$
<i>Cortinarius bivelus</i>	$p<0.005^*$	<i>Strobilomyces strobilaceus</i>	$p<0.06$
<i>Russula vesca</i>	$p<0.01^*$	Boletales unidentified	$p<0.05$
<i>Paxillus involutus</i>	$p<0.001^*$	<i>Phylloporus leucomycelinus</i>	$p<.10$
<i>Hydnum repandum</i>	$p<0.01^*$		
<i>Cortinarius violaceus</i>	$p<0.04^*$		
<i>Clavulina cinerea</i>	$p<0.04^*$		
<i>Hydnum repandum</i>	$p<0.01^*$		
<i>Cortinarius violaceus</i>	$p<0.04^*$		
<i>Clavulina cinerea</i>	$p<0.04^*$		

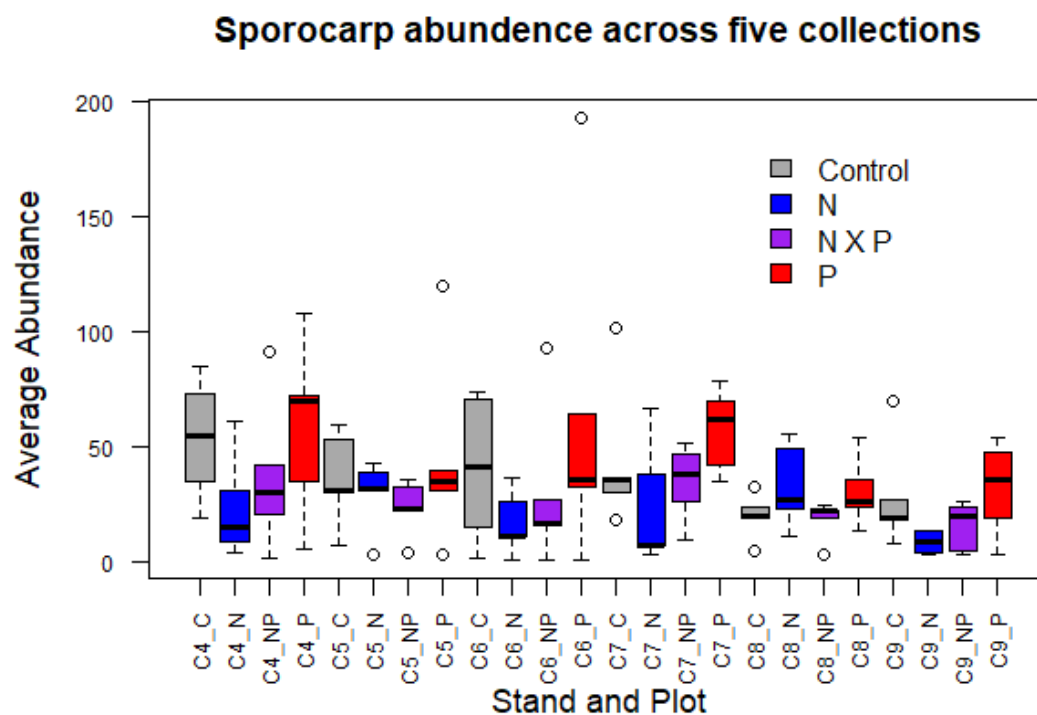
## Sporocarp Abundance, Biomass, and Species Richness

EMF responded to changes in nutrient conditions (Figure 6). Sporocarp abundance was significantly lower in plots treated with N ( $p=0.0003$ ). The overall abundance of sporocarps was not impacted by P addition. There was also no significant effect of the interaction between N and P on fruiting abundance; reduced sporocarp production in plots treated with both N and P can be attributed to increased N availability. Two of the tree mature stands (C8 and C9) fruited significantly less abundantly ( $p=0.001$  and  $p=0.04$ , respectively), but there was no significant effect of stand age on fruiting abundance. Species richness was lower in N treated plots ( $p=0.01$ ) and was not significantly affected by the addition of P (Figure 7). There was no significant effect of the interaction of N and P on sporocarp species richness.

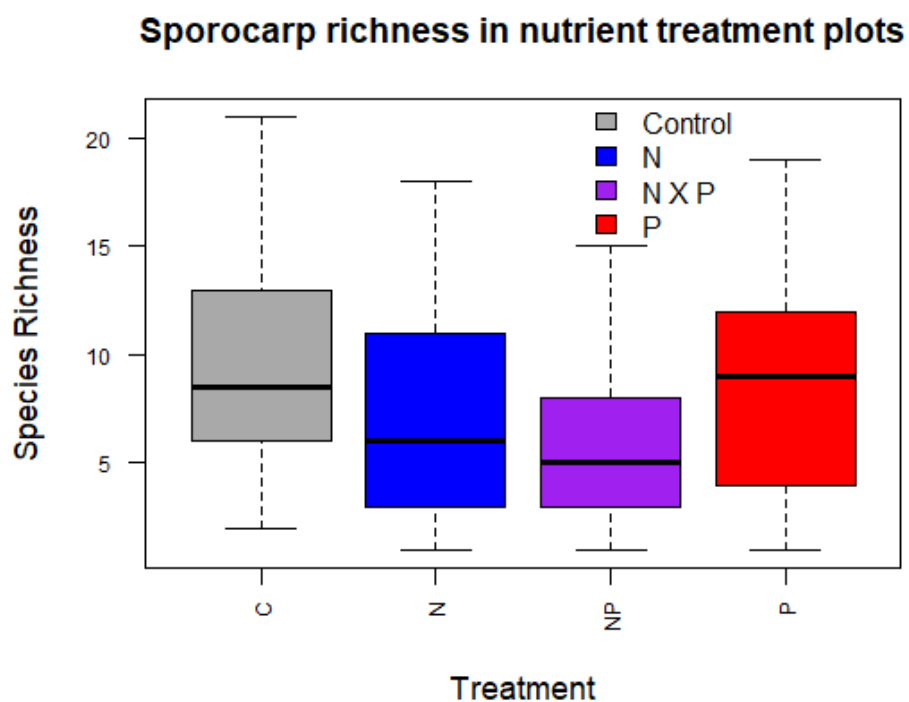
Sporocarp biomass was not significantly impacted by any factor besides the basal area of ectomycorrhizal trees ( $p=0.007$ ). Interestingly, the basal area of ectomycorrhizal trees did not impact fungal abundance but did positively impact aboveground fungal biomass. Increased basal area of red oak ( $p=0.02$ ) and white birch ( $p=0.04$ ) positively impacted overall EM sporocarp biomass.

**Table 9:** Analysis of variance table for the response ‘abundance’. Run with the Kenward-Roger approximation for the denominator degrees of freedom.

Source of variation	Numerator DF	Denominator DF	F value	Pr(>F)
Stand (Block)	5	15.0	3.07	0.0418
N addition	1	15.0	21.27	0.0003
P addition	1	15.0	2.91	0.1089
N addition: P addition	1	15.0	0.09	0.7722
Trip	4	79.3	16.09	1.10E-09
Trip : N addition	4	79.3	0.51	0.7252
Trip : P addition	4	79.3	1.00	0.4111
Trip : N addition : P addition	4	79.3	0.25	0.9094



**Figure 6:** Average abundance in each sampled plot across five collections. Fruiting abundance was suppressed in plots treated with nitrogen ( $p=0.0003$ ). Error bars are standard error of the mean.



**Figure 7:** Average species richness in plots across four nutrient treatments. Species richness was suppressed in plots with added N ( $p=0.01$ ). Error bars are standard error of the mean.

## DISCUSSION

### **Sporocarp Community Composition**

Community analyses support my first hypothesis that EMF sporocarp community composition would respond to nutrient additions (Figure 5). Sporocarp community composition was dissimilar in plots treated with either N or P relative to control. Further, when both N and P were added to a plot EMF fruiting composition was more dissimilar from control plots than when either nutrient was added alone. This result suggests an additive effect of the two nutrients on overall fruiting composition. Almeida *et al.* (2019) demonstrated corroborating results of EMF soil community composition. They found that while communities in plots treated with ammonium nitrate (N) were different from communities in control plots, this effect was less drastic relative to the community shift between plots treated with both N and superphosphate (P) relative to control (Almeida *et al.* 2019). They also reported dissimilar belowground fungal communities in control and P plots but did not indicate P plots as intermediate to NP plots in the same way as N plots (Almeida *et al.* 2019). These results suggest that both above and belowground fungal community composition changes with changing nutrient conditions. A greater shift in the nutrient condition of a forest stand, or a greater increase in nutrient availability, seems to cause a greater shift in EM fungal sporocarp production as well as belowground community composition.

Seasonal changes in sporocarp composition reflect changes in environmental conditions across the fruiting season. The average weekly temperature dropped during the last two collection trips (Table 4) and leaf senescence occurred between the fourth and fifth trips. The assemblage of sporocarps collected in each collection trip was different across time (Figure 3). Fungal sporocarp production is stimulated by a range of environmental factors including temperature and rainfall (Gange *et al.* 2007; Boddy *et al.* 2010). Sporocarp community composition in the middle of the



fruiting season (mid-August and early September) responded to nutrient additions, but this response was not detectable in NMDS plots at the beginning or end of the fruiting season (Figure 4). Variation in fungal community responses to nutrient additions throughout the season may be caused by seasonal cycles in tree storage and transport of nonstructural carbohydrates (NSC). NSC and N are mobilized from perennial tree storage pools such as branches and coarse roots to support tissue growth and respiration during the early growing season and pools are replenished when tree growth ceases (Hoch *et al.* 2003; Mei *et al.* 2015). These changes throughout the season likely impact carbon availability belowground (Horowitz *et al.* 2009), and subsequently, sporocarp production.

While belowground community composition indicates important responses to nutrient changes such as mycorrhizal formation and hyphal biomass, these responses only provide one indication of the fungal condition and are detectable less quickly than sporocarp responses (Peter *et al.* 2001). Analyzing sporocarp production at multiple points throughout a fruiting season may provide a clearer picture of how nutrient responses shift along with seasonal climatic conditions. In addition to soil and root sampling, sporocarp collection provide an additional proxy for understanding EM responses to nutrient additions.

### **Indicator Species**

Agerer (2006) indicated important differences in hyphal growth patterns of different ectomycorrhizal taxa including rhizomorph presence, the hydrophobicity of rhizomorphs, and hyphal exploration type. Hobbie and Agerer (2010) connected N responses of ectomycorrhizal taxa to hyphal growth strategies and indicated patterns regarding which genera may respond to changes in N conditions. Their results suggest that fungi with high biomass exploration type

hyphae that produce N mobilizing enzymes are likely well adapted to mine soil nutrients, and are more likely to be suppressed in high N environments (Hobbie and Agerer 2010). Lilleskov *et al.* (2002) indicated ‘nitrophilic’, or ‘high N’ taxa, and ‘nitrophobic’, or ‘low N’ taxa based on how well-studied taxa have responded to N additions. ‘Low N’ taxa include *Cortinarius* spp., *Russula* spp., *Piloderma croceum* (group), *Tricholoma inamoenum*, *Suillus variegatus*, and *Suillus bovinus*. ‘High N’ taxa include *Lactarius theiogalus*, *Lactarius rufus*, *Paxillus involutus*, *Laccaria bicolor*, and *Thelephora terrestris*. Although this list is not exhaustive of all ectomycorrhizal species, these classifications may improve our ability to predict how functionally similar taxa may respond to N additions. Further, these classifications provide an ecological context for understanding why some fungal groups may be sensitive to nutrient additions.

Results from my indicator species analyses contribute to a growing body of knowledge on mycorrhizal species level responses to nutrient additions. The response of sporocarp production of specific taxa to P additions are amongst the first evidence of differing P effects on EM fungal species (Table 7). Species that fruited more abundantly in plots without added nutrients and can be considered ‘Low N’ and ‘Low P’ taxa. These groups may be sensitive to both N and P additions. Species within the order Boletales fruited more abundantly in control plots but there is no overlap with the Boletales species previously indicated by Lilleskov *et al.* (2002) as ‘nitrophobic’, though this list is not exhaustive. Different species within the genus *Amanita* were significantly more abundant in control, N addition, and P addition plots, suggesting that there is variation in nutrient responses, and perhaps the functional roles, that different *Amanitas* play in ecosystems. *Paxillus involutus* is the only species significantly more abundant with both N and P additions, but this result was not statistically significant. The genera *Cortinarius* and *Russula* have been shown to decrease in abundance following N deposition (Lilleskov *et al.* 2002). Three species within the

genus *Cortinarius* fruited more abundantly in plots where P was added and N was not, although these results were not significant. An unidentified *Russula* species was the only species that fruited significantly more abundantly in P addition plots. Fungi in the genus *Lactarius*, which is classified as having ‘contact’ type hyphae (Agerer 2001), made up a significant portion of the sporocarps counted from the BEF, but did not respond significantly to changes in nutrient conditions. This may indicate the *Lactarius* species collected in this project are resilient to added N and P. Gorissen *et al.* (1991) exposed seedlings of *Pseudotsuga menziesii* in symbiosis with *Lactarius* to  $(\text{NH}_4)_2\text{SO}_4$  corresponding to up to 200 kg N ha<sup>-1</sup> and found no adverse effects by N on mycorrhizal frequency. Responses of species within the family Russulaceae (including *Russula* and *Lactarius*) to N additions vary (Lilleskov *et al.* 2011). Fungal responses to nutrient additions may be correlated with a species’ capacity to mine and transport limiting nutrients from soil so community assemblage structure in areas exposed to high nutrient deposition may shift away from these species. Understanding indicator species of different nutrient addition plots provides deeper insight to understand how fungal community assemblages change in response to nutrient pollution.

### **Sporocarp Abundance, Biomass, and Species Richness**

EMF sporocarp abundance and species richness were suppressed in N addition plots (Figures 6 and 7). Sporocarp biomass did not respond to changes in N and was affected only by the basal area of host trees, and specifically the basal area of red oak and white birch. N responses between biomass and sporocarp abundance may differ because the dataset used to analyze biomass results was considerably smaller than the whole dataset. While community composition was different in plots with added P, this did not correspond to an overall change in fruit body abundance, biomass, or species richness.

Reduced sporocarp production may indicate reduced allocation of carbon by trees to EMF (Högberg *et al.* 2010). However, this response may also be an indication of salt stress from the nitrogen fertilizer. Increased N availability is associated with decreased belowground carbon allocation and has led to a decline in the abundance and species richness of EMF taxa both above and belowground (Lilleskov *et al.* 2002a; Tresender 2004; Lilleskov *et al.* 2011). Relative to the response of sporocarp productivity, belowground fungal community composition responds less immediately, and is ultimately less drastic after N addition (Peter *et al.* 2001). A reduction in sporocarp productivity following N additions indicates that the reproductive output of fungal species that specialize in N uptake may diminish when less carbon is being transferred to these fungi through mycorrhizal roots. Importantly, reductions in EMF abundance following ammonium nitrate deposition may also be related to added salt with fertilization, which can be problematic for EMF even in small amounts (Dixon *et al.* 1993). Responses of EMF abundance and species richness to phosphorus additions have been much less clear. Almeida *et al.* (2018) reported an increase in belowground fungal biomass when phosphorus limitation was alleviated via apatite additions, suggesting that phosphorus addition may stimulate fungal biomass of some species in P limited environments (Hagerberg *et al.* 2003; Almeida *et al.* 2018).

In BEF rhizosphere activity responded to N additions differently depending on whether rhizosphere soil was collected from trees associated with AM or EM fungi (Shan *et al.* 2018). In soils from EM associated trees, soil respiration and belowground fungal biomass decreased in N addition plots (Shan *et al.* 2018). A decrease in overall sporocarp abundance in N addition plots corroborates this result. Relative basal area increase (RBAI) however, increased following P additions in mid-aged and mature stands (Goswami *et al.* 2018). While RBAI was high in P addition plots amongst all EM associated tree species it varied among AM associated species (Goswami *et*

*al.* 2018). This suggests that EM symbioses may have mediated increased aboveground biomass production when P limitation was alleviated. If P additions alleviated a limitation, trees may have responded by transporting more carbon to mine for N, and therefore may support fungal species equipped to absorb and transport organic N in P addition plots.

## CONCLUSION

In summary, this project supports that EMF sporocarps respond to nutrient additions in northern hardwood forests. Nitrogen additions suppressed sporocarp species richness and abundance and dissimilar sporocarp assemblages were present in N plots and control plots. Phosphorus additions did not impact sporocarp abundance or species richness but significantly dissimilar sporocarp assemblages were found between P addition plots and control plots suggesting that fungal communities may shift without changing overall species richness or fruiting abundance. This result may indicate a shift towards ‘phosphophilic’ fungal taxa. Species significantly more common in P addition plots may be adapted to high P environments. This project indicated possible high-P or ‘phosphophilic’ species within *Russula* and *Cortinarius* but subsequent sampling of roots and soil hyphae should be done to further understand this response and to investigate indicator species among EMF that do not fruit above ground. Sporocarp production has been shown to respond more rapidly and more drastically to added nutrients than mycorrhizal root tips (Ritter and Tölle 1978; Peter *et al.* 2001). Sampling sporocarps biased my collection towards EMF that fruit above ground; this sampling method yields an incomplete picture of fungal species richness, as do alternative methods. The combined sampling of sporocarps, EM roots, and rhizosphere soils could provide the most robust evidence of nutrient effects on EMF species richness and community assemblage in sampled stands.

Some ectomycorrhizal fungi are sensitive to the nutrient condition of their environments. Mycorrhizal fungi absorb most of a plant's required soil nutrients (van der Heijden *et al.* 2008), but often mycorrhizal responses are left out of studies of forest nutrient dynamics. Measuring species level fungal responses to nutrient additions is paramount to understanding changes in forest ecosystem functions. Anthropogenic nutrient additions impact forest ecosystems. The sustained diversity of mycorrhizal fungi is important in mediating nutrient uptake and increasing the resiliency of forests to ecosystem change.

## Chapter 3: Conclusion and Reflections

The first chapter reviewed mycorrhizal symbioses and the current literature on the effect of nutrient limitations and additions on mycorrhizal fungi and mycorrhizae. Our understanding of how nutrients affect mycorrhizal fungi and how mycorrhizal fungi effect nutrient cycling is quickly becoming more established but major gaps remain in our knowledge. Observing fungal responses to change offers a unique challenge in part due to ambiguous morphological traits and the ephemeral nature of sporocarps. Very few measures of microbial responses to nutrient changes differentiate fungal responses, and those that do often group all fungal activity together rather than differentiating any species level responses. By sampling and identifying mycorrhizal fungi either as sporocarps, on roots, or in soil, we can observe species level nutrient dynamics and can better predict how different fungal species interact with their ecosystems.

Overall, N addition leads to a decreased reliance of trees on mycorrhizal associations, but this effect is not consistent across all fungal species and fungi most important in acquiring organic N seems to be the most sensitive to N deposition. The impact of phosphorus on mycorrhizal productivity, is even less clear. During my literature review I found sparse and contradicting reports of P effects on belowground fungal biomass and no reports of P effects on sporocarp production. Contradicting literature led to my hypothesis that P would not increase or decrease fruiting abundance but would correspond to a shift in dominant fungal communities. The functional role of different fungal species within an ecosystem may determine whether the species will respond to N addition positively, negatively, or not at all. Work mainly by Reinhard Agerer, Erik Hobbie, and Erik Lilleskov has begun to sort out individual species responses to changing N conditions but there is no such evidence for P adapted species.

Chapter two reports the results of my 2018 sporocarp survey in six MELNHE stands in BEF, New Hampshire. I provided a background on the ecology of EMF as well as information on the MELNHE project and what we know about the nutrient condition in MELNHE stands. My results provide additional information on indicator species of N addition and provide the first indications for the possibility of species associated with P addition. My hypothesis that overall sporocarp abundance and species richness would not change following P additions was supported. My results indicate a shift in sporocarp community composition in P treated plots. This response has not been reported in the literature although it is consistent with belowground community shifts in P treated plots reported by Almedia *et al.* (2018). Subsequent sampling of P addition plots could help determine if this response is consistent year after year.

An aspect of this project which could be seen as a limitation, is the fact that I sampled EMF as sporocarps and not fungi on roots or soil hyphae. It is important to note that these data answer questions about fungal productivity and the composition of fruiting epigeous fungi, not total EM fungal composition. Sampling sporocarps introduces a bias regarding which fungal groups are represented in my data. Most, but not all, mycorrhizal fungi produce sporocarps and of those that do not all species fruit aboveground. For the most part, hypogeous and resupinate fruiters are missing from my dataset. The field of molecular mycology is moving away from sporocarp, and even root collections, and towards soil sampling and high throughput sequencing. While these methods are powerful and answer important questions about which fungi are in an environmental sample, they do not negate the value of more traditional collections. Just like fruits forming on orchard trees, fungal fruiting is influenced by a host of environmental conditions and changes in the abundance and diversity of sporocarps likely indicates changes in the condition of an ecosystem's fungal, and microbial communities. The most thorough studies of fungal community



responses should sample roots, soil, and sporocarps to create the clearest possible picture of fungal species composition.

This thesis provides novel contributions to the fields of mycorrhizal and forest ecology:

- Contributions to the growing list of fungal species known to respond to N additions
- First indications of fungal species that may respond to P additions
- Indications of sporocarp fruiting community response to P additions and to N and P additions together.
- List of ectomycorrhizal species fruiting in MELNHE stands in BEF

While I am proud of the depth of my analysis, I see a range of possibilities for how these data could be used and for directions that this project could go in the future. Further analyses that could be carried out using these data, or thorough continued sampling are detailed below:

**Geospatial analysis of fungal types relative to host trees:** I collaborated with an ESF undergraduate student, Ben Furber, to begin a GIS analysis of fruiting productivity in MELNHE plots. We used heat maps developed in GIS to visualize sporocarp abundance in five MELNHE stands. These maps provide a visual representation of fruiting productivity of each plot after one full field season ranging from July - October. These maps can be broken down into a finer scale by plotting different fungal species within each MELNHE subplot (10x10 m area) and overlaying the MELHNE stem maps onto those maps. Statistical analyses of these maps could elicit results on how host tree location explains variation in fungal fruiting relative to nutrient additions.

**Genus and species level responses to N and P additions across multiple seasons:** Expanding the timeline of this project to include multiple seasons of collections would add a

degree of repeatability that is not realistic within the master's program timeline. If I had more field seasons to extend this project, I would repeat collections in each plot. Further, I would focus my collections into ecologically and taxonomically ambiguous families such as Hygrophoraceae or Clavulinaceae. These families may be underrepresented in the data because they are both morphologically challenging and ecologically ambiguous. Sporocarps from mycorrhizal genera such as *Ramaria*, *Clavulina*, and *Hygrophorus*, are morphologically similar to nonmycorrhizal genera such as *Ramariopsis*, *Clavulinopsis*, *Hygrocybe*, and *Hygrophoropsis*. Misidentifications of these and other genera in the field may have led to groups being underrepresented in the data. Continued collections informed by molecular identification methods could alleviate this potential bias.

## **Conclusion**

This thesis has defined my life for the past three years. What I have learned throughout this process will provide me a background to continue my career as a mycologist and as a scientist. My research has brought me to unanswered questions, and ESF has provided me with a skillset to begin to answer those questions. I am excited by the idea of connecting fungal functional traits with their responses to ecosystem pressures. I believe that community ecology can have predictive power to understand ecosystem responses to pressures if we understand the functional roles that members of that community have within their ecosystem. The rise of molecular approaches for the identification of ectomycorrhizal fungi has allowed us to see who is in the black box of fungal symbionts, but now we must push this question one step further to understand what each species is doing there. I am excited to contribute to the field of mycology through the use and development of novel molecular techniques to answer phylogenetic and ecological questions.

Through this project I have balanced field work with molecular work and have led undergraduate workers in both settings. I have successfully taught molecular techniques to undergraduates and have helped to teach my lab mates. I have learned how to use statistics to critically analyze a large dataset and have begun to understand programs used to analyze phylogenetic data. I hope to continue my education in a PhD to empower myself to answer outstanding questions about fungal ecology and to develop new questions of my own.

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## Appendix 1: R Code used for Analysis

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##### DATA ANALYSIS FOR MS THESIS #####

#THIS SCRIPT RERUNS ANALYSES USING CONFIRMED TAXONOMIC
ASSIGNMENTS#

##### IMPORT AND NAME THE FILES #####

#First the Environmental data frame
file.choose()

MelEnv<-
read.csv("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\MEL.ENV.3.2
5.2020.csv")

#Now the data matrix

MelAbund<-
read.csv("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\MEL.ABUND.
3.25.2020.csv")

#Now the Full Data Set

MelFull<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.3.25.2020.csv")))
MelFull_trt<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.3.25.2020.csv")))
MelFull_stand<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.3.25.2020.csv")))
Mel_NoTrip_stand<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel
_trips_added.csv")))
#Trip1 Only

MelFull1<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.1.csv")))

#Trip2 Only
```

```

MelFull2<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.2.csv")))

#Trip3 only

MelFull3<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.3.csv")))

#Trip4 only

MelFull4<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.4.csv")))

#Trip5 only

MelFull5<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
Full.5.csv")))

#Now the sp richness matrix

Mel_Rich<-
read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\Mel.
richness.csv")))
##### LOAD REQUIRED PACKAGES #####

library(vegan) #for life <3
install.packages("ade4")
install.packages("cluster")
library(ade4)
library(cluster)
install.packages("pvclust")
library(pvclust)
install.packages("ggplot2")
library(ggplot2) #for plotting
install.packages("car")
install.packages("ecodist") #for dissimilarity measures
library(lme4) #For linear mixed models
library(car) #For linear mixed models
library(ecodist)
install.packages("gclus")
library(gclus)
library(indicspecies)

```

```

install.packages("indicspecies")

#####Adding Color Blind Friendly Color Palette#####

cbbPalette <- c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442", "#0072B2",
"#D55E00", "#CC79A7")

##### STARTING SOME EXPLORATORY DATA ANALYSIS #####

MelAbund[1:5, 1:10]

head(MelAbund)
tabasco(MelAbund)
#looks good

nrow(MelAbund) #119
ncol(MelAbund) #115
range(MelAbund) #0-99

apply(MelAbund, 2, range)
ad <- table(unlist(MelAbund))
barplot(ad, las=1, xlab = "Abundance Class", ylab = "Frequency", col = gray(5 :0/5))
#Lots of zero values
#####

attributes(MelFull)
head(MelFull)

names(MelFull)

#####Making objects for analyses variables
N<-MelFull[,17]
P<-MelFull[,16]
Block<-MelFull[,14]
Stand_Age<-MelFull[,13]
Treatment<-MelFull[,15]
Trip<-MelFull[,12]
TripDate<- MelFull[,11]
EMTrees<- MelFull[,10]
Sporo.Matrix<-MelFull[,18:132]
total_abund<-MelFull[,133]
Sporo.Env<- MelFull[,1:18]

#Species matrices
SpeciesMatrix_tot<-decoStand(Sporo.Matrix,method="total")
SpeciesMatrix_max<-decoStand(Sporo.Matrix,method="max")

```

```

SpeciesMatrix_wis<-wisconsin(Sporo.Matrix)

#making a dissimilarity matrix with mel_tot
mel_dist<- vegdist(SpeciesMatrix_tot)

#####NMDS#####

SpeciesNMDS<-metaMDS(SpeciesMatrix_wis, distance = "bray", k= 3, choices = c(1,3), try =
100, trymax = 100) #Generate NMDS. Use which ever matrix you want from above

stressplot(SpeciesNMDS) #Stress plotplot(Matrix_World_Bray) #Plotting roughly
?stressplot
plot(SpeciesNMDS, "sites", main = "All Trips Ordination")
mel_ellip<-ordiellipse(SpeciesNMDS, Treatment, col=c("black", "blue", "purple", "red"),
label=T, main = "Treatment NMDS")

NMDS1<-SpeciesNMDS$points[,1]    #Creating an object from the NMDS1
NMDS2<-SpeciesNMDS$points[,2]    #Creating object from NMDS2

DataForSpeciesNMDSPlot<-data.frame(Nitrogen=N, Phosphorus=P, Block=Block,
Treatment=Treatment, NMDS1=NMDS1, NMDS2=NMDS2)

#Treatment plot ##call different axis look in help page
SpeciesNMDS_Treatment<-ggplot(DataForSpeciesNMDSPlot, aes(x=NMDS1, y=NMDS2,
col=Treatment)) +
  geom_point(size = 1.5) + theme_classic()+
  stat_ellipse(level=.49) +
  theme(legend.key.size = unit(.45, "cm"), legend.text = element_text(size=9), legend.title =
element_text(size=12), legend.position = c(.875, 0.2), legend.key = element_rect(fill = "white"))
+ scale_color_manual(values=c("black", "blue", "purple", "red"))
SpeciesNMDS_Treatment

#Trip Plot -> Looking good
SpeciesNMDS_Trip<-ggplot(DataForSpeciesNMDSPlot, aes(x=NMDS1, y=NMDS2,
col=TripDate)) +
  geom_point(size = 1.5) + theme_classic()+
  stat_ellipse(level=.49, size = 1) +
  ggtitle("Sporocarp Community Ordination by Season") +
  theme(legend.key.size = unit(.45, "cm"), legend.text = element_text(size=9), legend.title =
element_text(size=12), legend.position = c(.875, 0.2), legend.key = element_rect(fill = "white"))
+
  scale_colour_manual(values=c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442",
"#0072B2"),breaks=c("LateJuly", "MidAugust", "EarlySeptember", "LateSeptember",
"MidOctober"))
SpeciesNMDS_Trip

```

##### CONSTRAINING THE ORDINATION FOR THE TREATMENT PLOT #####

#constrained ordination, plotting in ggplot #does this remove zeros?

head(MelFull)

?capscale

SpeciesCap<- capscale(SpeciesMatrix\_tot~MelFull\$str, distance="bray")

SpeciesCap\_scores<- scores(SpeciesCap, choices= c(1,2))

Cap1<-SpeciesCap\_scores\$sites[,1]

Cap2<-SpeciesCap\_scores\$sites[,2]

DataForSpeciesCapPlot<-data.frame(N=N, P=P, Block=Block, Treatment=Treatment,  
Stand\_Age=Stand\_Age, TripDate=TripDate, Cap1=Cap1, Cap2=Cap2)

#CAP plot x Trt -> Looks good

SpeciesCap\_Treatment<-ggplot(DataForSpeciesCapPlot, aes(x=Cap1, y=Cap2, col=Treatment))

+ geom\_point(size = 1.5) + theme\_classic()+

stat\_ellipse(level=.40, size = 1) +

ggtitle("Sporocarp Community Ordination by Nutrient Treatment") +

theme(legend.key.size = unit(.45, "cm"), legend.text = element\_text(size=9), legend.title =  
element\_text(size=12), legend.position = c(.875, 0.2), legend.key = element\_rect(fill =  
"grey92")) +

scale\_color\_manual(values = c("black", "blue", "purple", "red"))

plot(SpeciesCap\_Treatment)

anova(SpeciesCap, by= "axis")

#####

##### Trip By Trip Ordinations #####

#####Trip 1#####

names(MelFull1)

#making objects

#####Making objects for analyses variables

N<-MelFull1[,17]

P<-MelFull1[,16]

Block<-MelFull1[,14]

Stand\_Age<-MelFull1[,13]

Treatment<-MelFull1[,15]

Trip<-MelFull1[,12]

TripDate<- MelFull1[,11]

```

EMTrees<- MelFull1[,10]
Sporo.Matrix1<-MelFull1[,18:132]

#Species matrices
SpeciesMatrix1_tot<-decostand(Sporo.Matrix1,method="total")
SpeciesMatrix1_max<-decostand(Sporo.Matrix1,method="max")
SpeciesMatrix1_wis<-wisconsin(Sporo.Matrix1)

#making a dissimilarity matrix with mel_tot
mel_dist<- vegdist(SpeciesMatrix1_max)

#NMDS
SpeciesNMDS1<-metaMDS(SpeciesMatrix1_wis, distance = "bray", K=2, try = 50, trymax =
50) #Generate NMDS. Use which ever matrix you want from above
stressplot(SpeciesNMDS1) #Stress plotplot(Matrix_World_Bray) #Plotting roughly

plot(SpeciesNMDS1, "sites", method = "bray", main = "Trip 1 Ordination by Treatment")

mel_ellip<-ordiellipse(SpeciesNMDS1, Treatment, col=c("black", "blue", "purple", "red"),
label=T, main = "Treatment NMDS Trip 1")
legend(locator(1), lwd = 2, col = c("black", "blue", "purple", "red"), legend = c("Control", "N",
"N x P", "P"), bty = "n")

#####Trip 2#####

names(MelFull2)

N<-MelFull2[,17]
P<-MelFull2[,16]
Block<-MelFull2[,14]
Stand_Age<-MelFull2[,13]
Treatment<-MelFull2[,15]
Trip<-MelFull2[,12]
TripDate<- MelFull2[,11]
EMTrees<- MelFull2[,10]
Sporo.Matrix2<-MelFull2[,18:132]

#Species matrices
SpeciesMatrix2_tot<-decostand(Sporo.Matrix2,method="total")
SpeciesMatrix2_max<-decostand(Sporo.Matrix2,method="max")
SpeciesMatrix2_wis<-wisconsin(Sporo.Matrix2)

#making a dissimilarity matrix with mel_tot

```

```

mel_dist<- vegdist(SpeciesMatrix2_max)

#NMDS
SpeciesNMDS2<-metaMDS(SpeciesMatrix2_wis, k=2, distance = "bray", try = 50, trymax = 50)
#Generate NMDS. Use which ever matrix you want from above
stressplot(SpeciesNMDS2) #Stress plotplot(Matrix_World_Bray) #Plotting roughly

plot(SpeciesNMDS2, "sites", method = "bray", main= "Trip 2 Ordination by treatment")

mel_ellip<-ordiellipse(SpeciesNMDS2, Treatment, col=c("black", "blue", "purple", "red"),
label=T, main = "Treatment NMDS Trip 1")
legend(locator(1), lwd = 2, col = c("black", "blue", "purple", "red"), legend = c("Control", "N",
"N x P", "P"), bty = "n")

#####Trip 3#####

names(MelFull3)
head(MelFull3)

N<-MelFull3[,17]
P<-MelFull3[,16]
Block<-MelFull3[,14]
Stand_Age<-MelFull3[,13]
Treatment<-MelFull3[,15]
Trip<-MelFull3[,12]
TripDate<- MelFull3[,11]
EMTrees<- MelFull3[,10]
Sporo.Matrix3<-MelFull3[,18:132]

#Species matrices
SpeciesMatrix3_tot<-decostand(Sporo.Matrix3,method="total")
SpeciesMatrix3_max<-decostand(Sporo.Matrix3,method="max")
SpeciesMatrix3_wis<-wisconsin(Sporo.Matrix3)

#making a dissimilarity matrix with mel_tot
mel_dist<- vegdist(SpeciesMatrix3_max)

#NMDS
SpeciesNMDS3<-metaMDS(SpeciesMatrix3_wis, distance = "bray", k= 2, try = 50, trymax =
50) #Generate NMDS. Use which ever matrix you want from above
stressplot(SpeciesNMDS2) #Stress plotplot(Matrix_World_Bray) #Plotting roughly

plot(SpeciesNMDS3, "sites", method = "bray", main= "Trip 3 Ordination by Treatment")

```



```

mel_ellip<-ordiellipse(SpeciesNMDS3, Treatment, col=c("black", "blue", "purple", "red"),
label=T, main = "Treatment NMDS Trip 1")
legend(locator(1), lwd = 2, col = c("black", "blue", "purple", "red"), legend = c("Control", "N",
"N x P", "P"), bty = "n")

#####Trip 4#####

names(MelFull4)
head(MelFull4)

N<-MelFull4[,17]
P<-MelFull4[,16]
Block<-MelFull4[,14]
Stand_Age<-MelFull4[,13]
Treatment<-MelFull4[,15]
Trip<-MelFull4[,12]
TripDate<- MelFull4[,11]
EMTrees<- MelFull4[,10]
Sporo.Matrix4<-MelFull4[,18:132]

#Species matrices
SpeciesMatrix4_tot<-decostand(Sporo.Matrix4,method="total")
SpeciesMatrix4_max<-decostand(Sporo.Matrix4,method="max")

SpeciesMatrix4_wis<-wisconsin(Sporo.Matrix4)

#making a dissimilarity matrix with mel_tot
mel_dist<- vegdist(SpeciesMatrix4_max)

#NMDS
SpeciesNMDS4<-metaMDS(SpeciesMatrix4_wis, distance = "bray", k=2, try = 50, trymax = 50)
#Generate NMDS. Use which ever matrix you want from above
stressplot(SpeciesNMDS4) #Stress plotplot(Matrix_World_Bray) #Plotting roughly

plot(SpeciesNMDS4, "sites", method = "bray", main= "Trip 4 Ordination by Treatment")

mel_ellip<-ordiellipse(SpeciesNMDS4, Treatment, col=c("black", "blue", "purple", "red"),
label=T)
legend(locator(1), lwd = 2, col = c("black", "blue", "purple", "red"), legend = c("Control", "N",
"N x P", "P"), bty = "n")

#####Trip 5#####3

names(MelFull5)
head(MelFull5)

```

```

N<-MelFull5[,17]
P<-MelFull5[,16]
Block<-MelFull5[,14]
Stand_Age<-MelFull5[,13]
Treatment<-MelFull5[,15]
Trip<-MelFull5[,12]
TripDate<- MelFull5[,11]
EMTrees<- MelFull5[,10]
Sporo.Matrix5<-MelFull5[,18:132]

#Species matrices
SpeciesMatrix5_tot<-decostand(Sporo.Matrix5,method="total")
SpeciesMatrix5_max<-decostand(Sporo.Matrix5,method="max")
SpeciesMatrix5_wis<-wisconsin(Sporo.Matrix5)

#making a dissimilarity matrix with mel_tot
mel_dist<- vegdist(SpeciesMatrix5_max)

#NMDS
SpeciesNMDS5<-metaMDS(SpeciesMatrix5_tot, distance = "bray", k=2, try = 50, trymax = 50)
#Generate NMDS. Use which ever matrix you want from above
stressplot(SpeciesNMDS5) #Stress plotplot(Matrix_World_Bray) #Plotting roughly

plot(SpeciesNMDS5, "sites", method = "bray", main = "Trip 5 Ordination by Treatment")

mel_ellip<-ordiellipse(SpeciesNMDS5, Treatment, col=c("black", "blue", "purple", "red"),
label=T)
legend(locator(1), lwd = 2, col = c("black", "blue", "purple", "red"), legend = c("Control", "N",
"N x P", "P"), bty = "n")

#####Exploring indicator species #####

vignette("indicspeciesTutorial", package="indicspecies")
library(indicspecies)

names(Mel_NoTrip_stand)

stand_age
Age<-Mel_NoTrip_stand[,1]
trt<-Mel_NoTrip_stand[,3]
stand<- Mel_NoTrip_stand[,2]
matrix<-Mel_NoTrip_stand[,6:120]

as.factor(trt)
as.factor(stand)

```

```
#####Indicator species by treatment. Two different appraoches.

####
#Using restcomb to specify each treatment group and specific combinations of treatment groups.
Here I did the main groups plus N+NP, and P+NP. But, power is low.

Mel_Indic_trt2 <- multipatt(matrix, Age, control = how(nperm =9999),restcomb=c(1,2,3,4,8,14))
summary(Mel_Indic_trt2, indvalcom = TRUE, minstat=.1, alpha = .3)

#####Indicator species by Stand or Age#####

Mel_Indic_Age1 <- multipatt(matrix, Age, control = how(nperm =9999),duleg=TRUE)
summary(Mel_Indic_Age1, indvalcom = TRUE, minstat=.1, alpha = 1)
##### outputs are saved in Notepad#####

#####

library(lmerTest)

#####
#####
#Variables for modling
Trip.fact<-as.factor(MelFull$trip)
MelFull$stand
MelFull$P_added
MelFull$N_added
MelFull$stand_trt #Or use the variable I created above, MelFull$StandPN. It produces the same
results.
MelFull$total_abund
MelFull$stand_age
MelFull$trip
MelFull$BasalAreaEMTrees

Trip.fact<-as.factor(Mel_Rich$trip)
Mel_Rich$stand
Mel_Rich$P_added
Mel_Rich$N_added
Mel_Rich$total_abund
Mel_Rich$stand_age
Mel_Rich$trip
Mel_Rich$BasalAreaEMTrees
Mel_Rich$sp_richness
Mel_Rich$trt
#####
# The last part with the 1| is the random effect
```

```
Abundance_Model1<-lmer(sqrt(total_abund) ~ Trip.fact*P_added*N_added + stand +  
(1|stand_trt), data= MelFull) #The main model. I sqrt transformed it to better meet the  
assumptions.
```

```
summary(Abundance_Model1) #Model summary  
hist(resid(Abundance_Model1)) #Inspecting normality. Good enough  
qqnorm(resid(Abundance_Model1)) #Inspecting normality  
qqline(resid(Abundance_Model1)) #Inspecting normality.  
fligner.test(MelFull$total_abund~MelFull$stand_trt) #Tests variance assumption. Good.
```

```
anova(Abundance_Model1, type=3, ddf="Kenward-Roger") # Testing with the kenward-Roger  
approximation for the denominator degrees of freedom.
```

```
plot(MelFull$stand_trt, MelFull$total_abund, las =2, ylab= "Average Abundance", xlab= "Stand  
and Plot", main= "Sporocarp abundance across five collections", cex.lab=1.15, cex.axis=0.75,  
col=c("darkgrey", "blue", "purple", "red"))
```

```
legend(locator(1), fill = c("darkgrey", "blue", "purple", "red"), legend = c("Control", "N", "N X  
P", "P"), bty = "n")
```

```
Abundance_Model2<-lmer(sqrt(richness) ~ TripCat*P_added*N_added + stand + (1|stand_trt),  
data= MelFull) #The main model. sqrt transformed to better meet the assumptions.
```

```
summary(Abundance_Model2) #Model summary  
hist(resid(Abundance_Model2)) #Inspecting normality. Good enough  
qqnorm(resid(Abundance_Model2)) #Inspecting normality  
qqline(resid(Abundance_Model2)) #Inspecting normality.  
fligner.test(MelFull$total_abund~MelFull$stand_trt) #Tests variance assumption. Good.
```

```
anova(Abundance_Model2, type=3, ddf="Kenward-Roger") # Testing with the kenward-Roger  
approximation for the denominator degrees of freedom.
```

```
plot(Mel_Rich$trt, Mel_Rich$sp_richness, las =2, ylab= "Species Richness", xlab= "Treatment",  
main= "Sporocarp richness in nutrient treatment plots", cex.lab=1.15, cex.axis=0.75,  
col=c("darkgrey", "blue", "purple", "red"))  
legend(locator(1), fill = c("darkgrey", "blue", "purple", "red"), legend = c("Control", "N", "N X  
P", "P"), bty = "n")
```

```
##### B2 Biomass analysis for thesis #####
```

```
B2<read.csv((as.matrix("C:\\Users\\cvict\\OneDrive\\Documents\\FinalDataMatricesMELNHE\\  
B2_matrix_notrip.csv")))
```

```
names(B2)
```

```
N<-B2[,4]
```

```

P<-B2[,5]
stand<-B2[,2]
EMtrees<-B2[,1]
trt<-B2[,3]
matrix<- B2[,6:68]
totalmass<-B2[,69]
BA<-B2[,]

matrix_relCol2<-(decostand(matrix,method="max",margin=2, na.rm = TRUE)) #Relativizing
values to be a proportion of the total count in a sample.

adonis(matrix_relCol2 ~ EMtrees + N + P+ P*N + stand, method="bray", data= B2)

B2$stand
B2$Pyes
B2$Nyes
B2$stand_age
B2$BA_EMTrees
B2$Grand.Total
B2$trt
B2$BA
B2$BASS
B2$BE
B2$HEM
B2$QA
B2$RO
B2$RS
B2$WB
B2$YB
#####
Abundance_Model3<-lmer((Grand.Total) ~ EMtrees + P_added*N_added + stand + (1|stand),
data= B2)

summary(Abundance_Model3)
hist(resid(Abundance_Model1))
qqnorm(resid(Abundance_Model1))
qqline(resid(Abundance_Model1))
fligner.test(MelFull$total_abund~MelFull$stand_trt)

anova(Abundance_Model3, type=3, ddf="Kenward-Roger")

plot(B2$trt, B2$Grand.Total, las =2, ylab= "Total Biomass", xlab= "Treatment", main= "Total
fruit body biomass", cex.lab=1.15, cex.axis=0.75, col=c("darkgrey", "purple", "blue", "red"))

```

# Curriculum Vitae

Claudia Victoroff  
Curriculum Vitae

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Syracuse, NY 13210

## EDUCATION AND HONORS

---

<b>State University of New York</b>	2017 – present
<b>College of Environmental Science and Forestry (SUNY ESF)</b>	
Master of Science (in progress)	
Area of Study: Mycology and Forest Pathology	
Current GPA: 3.655	
<b>Loyola University of Chicago (LUC) <i>Magna Cum Laude</i></b>	2013 – 2017
Bachelor of Science	
Major: Environmental Science	
Minor: English	
Concentration: Food Systems and Sustainable Agriculture	
GPA: 3.832	

## EMPLOYMENT

---

<b>SUNY ESF Dept. of Environmental and Forest Biology, Syracuse NY</b>	
Graduate Teaching Assistant	
<i>EFB 320 General Ecology Lab</i>	Fall 2017, 2018, 2019
- Instructed two laboratory sections to conduct, analyze, and write independent research projects	
- Held weekly office hours to support student comprehension of ecological concepts	
<i>EFB 120 The Global Environment and the Evolution of Human Society</i>	Spring 2019
- Guided undergraduate students in small group projects integrated within their communities	
- Facilitated group discussions on environmental issues and their relation to human societies	
<i>Graduate Research Assistant</i>	Spring 2018, Spring 2019 – present
- Utilized aseptic technique and molecular approaches to produce and identify fungal cultures	
Cranberry Lake Biological Station	
<i>Field Instructor</i>	Summer 2019
- Developed and taught a course on field mycology and fungal ecology	
MELNHE Project (SUNY ESF)	
<i>Field Researcher</i>	Summer 2017, Summer and Fall 2018
- Lived and worked with a group of 15 field researchers to study forest nutrient responses in the White Mountains, NH. Planned and executed research on fungal communities in nutrient manipulated forest stands.	
<b>Loyola University of Chicago Institute of Environmental Sustainability</b>	
<i>Undergraduate Researcher</i>	2016 – 2017

- Designed and conducted an experiment to analyze collected dust samples from the roofs of DePaul and Loyola Chicago buildings for the presence of mycorrhizal fungal spores. Isolated and morphotyped spores. Data was analyzed in R Studio and presented research for undergraduate thesis.
- Designed and conducted an experiment with a small group to determine the variability of waste products from beer and coffee production as a sustainable and economical substrate for the propagation of edible mushrooms.

#### *Undergraduate Research Assistant*

2016

- Quantified mycorrhizal structures in roots under a compound microscope. Analyzed data in R Studio to determine the effectiveness of mycorrhizal inoculation of green roof soils.

### **AWARDS AND COMPETITIVE GRANTS FUNDED**

---

Lowe Wilcox Scholarship. \$11,500 total.	2017, 2018, 2019
SUNY ESF Graduate Student Association Travel Grant. \$250.	2018
SUNY ESF Graduate Student Association Travel Grant. \$356.	2019
SUNY ESF Graduate Student Employees Union Professional Development Award. \$400.	2018
Edna B. Sussman Foundation Graduate Intern. \$7,500.	2017
Mulcahy Fellowship, Loyola University College of Arts and Sciences. \$2,000.	Spring 2016
Member, National Society of Collegiate Scholars	2014 – present
Institute of Environmental Sustainability Director's Academic Excellence List	2015 – 2017
College of Arts and Science Dean's List	2013 – 2014

### **ORAL AND POSTER PRESENTATIONS**

---

- Victoroff, C.V., Horton, T.R., Yanai, R.D. – Fruiting response of ectomycorrhizal fungi to nutrient additions in Bartlett Experimental Forest, New Hampshire. Mycological Society of America Meeting. Minneapolis MN. August 2019.
- Victoroff, C.V., Giovati A.S., Hilmarsdóttir-Puetzer N.A.E, Ruschmeyer, S., Whalen, C., Horton, T.R. – Initial analysis of ectomycorrhizal sporocarp production in nutrient manipulated stands in Bartlett, NH. MassMyco. Boston, MA. October 2018
- Victoroff, C.V., Potter, G., Castracane, J., Horton, T.R. – Investigating resource allocation by *Pinus strobus* seedlings inoculated with three mycorrhizal symbionts: *Suillus brevipes*, *Rhizopogon cf. rubescens*, and *Phiolocephala fortinii* 11<sup>th</sup> International Mycological Congress. San Juan, PR. July 2018.
- Victoroff, C.V., T.R. Horton, R.D. Yanai. – Fruiting response of ectomycorrhizal fungi to nutrient additions in Bartlett Experimental Forest, NH. Northeast Natural History Conference. Springfield, MA. April 2019
- Victoroff, C.V., Giovati A.S., Hilmarsdóttir-Puetzer N.A.E, Horton, T.R. – Fruiting response of ectomycorrhizal fungi to nutrient additions in northern hardwood forests. Hubbard Brook Collaborators Meeting, North Woodstock, NH. July 2019.
- Victoroff, C.N., Yanai, R.D., Horton, T.R. – Will Claudia find any mushrooms by mid-July? Hubbard Brook Collaborators Meeting, North Woodstock, NH. July 2017.
- Victoroff, C. N., Tomaka, C., Chaudhary, V.B., Ohsowski, B. – The distribution of arbuscular mycorrhizal spores by wind. Loyola University Chicago Weekend of Excellence. Chicago IL. April 2017.
- Victoroff, C. N. Stotz, T.A., and Ohsowski, B. – LUC Mycology: substrate analysis. Loyola University Chicago Climate Change Conference. Chicago, IL. March 2017

## EXTRACURRICULAR WORK AND VOLUNTEER EXPERIENCE

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### **Blaze Gymnastics**

*Coach* 2019 – present

### **Whole Foods Market Edgewater**

*Specialty Team Member* 2016 – 2017

### **George Jones Farm and Nature Preserve**

*Assistant Farm Manager* Summer 2016

*Assistant Education Director* Summer 2016

*Farm Intern* Summers 2014, 2015

### **Ohio Farm Direct**

*Vendor* Summers 2015 – 2018

### **LUC Department of Urban Agriculture**

*Student Intern* 2015 – 2016

### **LUC Restoration and Ecology Campus Student Farm**

*Student Worker* 2014 – 2015

### **First Flips and Elite Gymnastics Academy**

*Coach* 2011 – 2015

### **Syracuse Childcare Collective**

*Volunteer* 2017 – present

### **LUC Mycology Club**

*Vice President, founding member* 2015 – 2017

### **LUC Student Environmental Alliance**

*Campus Activities Network Representative* 2015 – 2016

*Member* 2013 – 2017

## EXPERTISE

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### *Lab Skills*

Production of fungal cultures; use of molecular approaches to identify fungi; edible mushroom propagation and management; use of aseptic technique; fungal morphotyping; use of compound and dissecting microscope

### *Field Skill*

Soil analysis using LI-COR; field navigation and leadership; outdoor safety, leadership, and education; plant propagation and management; identification and severity ranking of Beech Bark disease

### *Computer Skills*

Proficient in Microsoft Office 2007, 2010, 2013, 2016

Conversant in R Studio, SAS, SAS University, and QGIS